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FORMATIVE EFFECT OF HIGH AND LOW TEMPERATURES UPON GROWTH OF BARLEY: A CHEMICAL CORRELATION

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(WITH EIGHTEEN FIGURES)

Introduction

Cereals are commonly considered cool temperature crops. Cool seasons are known to favor cereal production, warm seasons to hinder cereal production. Physiologists have correlated these observations with the general effects of temperature upon the growth and maturation of the crop, but have given little attention to possible effects of the initial germination temperature upon the subsequent course of development of the plant. The investigation here reported is a study of the effects of high and low temperatures and concomitant variations in the supply of nitrogen, phosphorus, and potassium respectively upon the course of development of the barley plant. A chemical correlation has been established between temperature and nutrition effects.

Literature

ADERHOLD (1), working with young kohlrabi plants, noted that exposures of the young plants to temperatures of $-2°$ C. to $-8°$ C. for 8–10 hours tended to cause the plant to shoot into flowering instead of forming the desired "ball."

GUTZEIT (5) repeated ADERHOLD'S work and found by a rather extensive set of experiments that exposures to temperatures below zero had no effect on stem or shoot production in kohlrabi, beets, or various other plants. He did find, however, that a period at +4° C. during germination and early growth caused about 30 per cent of certain beets to produce shoots very early the first year. Some of the shoots produced only very short stems, and the plants were otherwise normal, while other shoots grew continuously and early produced flowers and seeds. Beets of exactly the same kind when kept at +22° C. during germination and early growth showed no shoot production the first year. Only such beets as were predisposed to early shoot production could be thus forced by low temperatures, so hereditary characters as well as temperature enter in as determining factors. GUTZEIT suggests that this temperature response explains why early seeding of beets causes much premature shoot production, whereas late seeding gives little or none. On the basis of other experiments conducted by himself, as well as data from the literature, GUTZEIT concludes that low temperatures during germination and early growth favor stem formation, while high temperatures at this time inhibit stem formation.

APPEL and GASSNER (2) noted in the experimental fields of summer cereals at the Agricultural Experiment Station at Dahlem, Germany, a peculiar sickness, the plants becoming light green, and the older leaves turning yellow. Since neither animal nor plant pests seemed to be attacking the cereals, an explanation for their condition was sought in unfavorable soil and weather relations. Greenhouse experiments conducted by APPEL and GASSNER led them to attribute the peculiar conditions of these summer cereals to a too high germination temperature.

They grew barley in pots in the greenhouse, keeping one lot at 20–25° C. and the other lot at 5–7° C. When the plants at the higher temperature had reached a height of 15 cm., those in the cool house had just come up. Both sets were then transferred to the open and kept under like conditions. After three weeks the barley plants from the warm house began to show signs of injury, the older leaves yellowing at their tips, and only the youngest leaves remaining green. The barley plants from the cool house

soon outstripped the high temperature plants, finally reaching twice the size. Figures of APPEL and GASSNER's plants show that there was an excessive leaf production and little stem production at the higher temperature. These investigations suggested that the light color of the leaves was due to nitrogen hunger, but they were unable to get any beneficial results from nitrogen fertilization. The addition of iron salts also had no favorable effect.

GASSNER (3) has made extensive observations and experimental studies upon the growth and development of cereals in subtropical climates, the experiments being carried out in the phytopathological experimental fields of the University of Montevideo, Uruguay. In considering the choice of varieties of summer cereals suitable for cultivation in Uruguay, he emphasizes the importance of temperature in the early stages of development, and suggests that decreased yields are often due to the lack of the necessary cold requirements (Kälteansprüche) in the early stages of growth. GASSNER quotes HELLRIEGEL (6) on the temperature relations of small 4-rowed barley. HELLRIEGEL maintained that in the first half of the vegetative period of the barley, the period of leaf and culm formation, an average daily temperature of about 15° C. is the best, whereas in the second half of the vegetative period, the period of head development and grain formation, a temperature of 17–18° C. is the most favorable. HELLRIEGEL therefore insists upon two different temperature optima in development of barley, the line of demarcation between the two optima being placed at the time of shooting.

GASSNER summarizes his views as follows (translated from the original article):

> We can therefore say that for winter cereals, as well as for summer cereals, the yield of a given variety of a cereal in a given climate is among other things dependent upon the influence of the climatic factors in the first stage of development in such a way that varieties of high "cold requirements" in their youth require a colder climate than varieties with lower "cold requirements," and that incomplete fulfillment of these requirements causes bad development and depression of the yield.

GASSNER states that the death and yellowing of the leaves of young plants previously described by APPEL and GASSNER rarely

occurs in Uruguay. He notes, however, that the culm habit in Uruguayan oats and rye germinated at high temperatures is distinctly recumbent, whereas it is upright from the beginning in the case of plants grown from seeds germinated at low temperatures. The low temperature plants begin the formation of the culm (shooting) much earlier than do the high temperature plants. A typical experiment with oats is outlined as follows:

Date of seeding	Temperature during germination	Date of transfer into field	Beginning shooting
January 18	January 18–23, 6–9°; January 23–25, 25°	January 25	March 15
January 18	January 18–20, 25°; January 20–25, 6–9°	January 23	No shoot formation on April 25, shooting not expected before October

In another series it was found that even 24 hours of exposure to a germination temperature of 25° led to the same abnormal course of development as indicated in the second series here quoted.

GASSNER and GRIMME (4) have made one attempt to correlate the effects of germination temperatures and the resistance of cereals to frost injury. They analyzed the first leaves of winter and spring rye germinated at 5–6° C. and at 28°. They found that the seedlings germinated at the lower temperature had a higher sugar content than seedlings germinated at the high temperature; moreover, seedlings of a hardy winter rye had a higher sugar content than those of a spring rye grown under the same conditions. Their results with rye are shown in table I.

HUTCHESON and QUANTZ (7) conducted experiments on the effect of greenhouse temperatures on the growth of the small grains: wheat, oats, barley, and rye. All four crops were grown under four temperature conditions, namely, 14.4° C., 16.6° C., 18.3° C., and 23.9° C. The higher temperature range had a distinctly detrimental effect upon the growth of the barley and a less harmful effect upon the growth of wheat and rye, while oats had a normal course of development at all the temperatures used, although the oat culms were weaker at the higher temperatures. The high

temperature barley plants showed an excessive development of tillers and no indication of ever heading. Inspection of the figures shows that the leaves of the high temperature plants were abnormally long, and especially so in the case of the barley. The general growth characters obtained by HUTCHESON and QUANTZ were obtained in the present investigation in the case of high temperature, high nitrogen series (fig. 13). These authors grew the grain

TABLE I

SUGAR CONTENT OF FIRST LEAVES OF RYE* (PERCENTAGE OF DRY WEIGHT)

SERIES NO.	TOTAL SUGAR	GERMINATION TEMPERATURE 5–6° C.		GERMINATION TEMPERATURE 28° C.		
		Reducing sugar	Non-reducing sugar	Total sugar	Reducing sugar	Non-reducing sugar
	Petkuser winter rye					
I	42.19	34.93	7.26	40.92	32.56	8.36
II	43.14	35.86	7.28	39.79	31.14	8.65
III	41.92	34.84	7.08	39.13	31.08	8.05
IV	42.31	35.85	6.46	40.73	33.94	6.79
V	40.97	32.31	8.66	39.52	34.11	5.41
	Petkuser spring rye					
I	36.58	29.41	7.17	31.57	27.13	4.44
II	37.08	30.57	6.51	33.26	26.58	4.68
III	35.39	30.41	4.98	32.59	26.81	5.78
IV	37.65	31.02	6.63	34.56	30.38	4.18
V	35.85	30.21	5.64	32.94	28.16	4.78

* Similar results were obtained with barley.

in 4-inch clay pots, two plants to the pot. No mention is made concerning the substrate used in their experiments.

This investigation of the influence of high and low temperatures upon the growth of barley was planned to ascertain in particular the influence of variations in the supply of nutrient salts with concomitant variations in the temperature. The nutrients varied were nitrogen, potassium, and phosphorus. Chemical analyses were made in order to relate certain observed differences in growth to possible differences in the chemical composition.

Method

Culture solutions

The method of sand culture was used throughout these experiments, the sand used being a highly pure Ottawa silica sand obtained from Ottawa, Illinois. Two gallon glazed stone jars were used as the culture vessels, each jar receiving 11.4 kilos of sand. The water content of each jar was maintained at approximately 13 per cent of the dry weight of the sand by means of frequent weighing. Tottingham's culture solution was used in diluted form. This solution has the following composition:

Solution A:
- KNO_3—0.0034 M (0.3437 gm. per liter)
- KH_2PO_4—0.0108 M (1.4692 gm. per liter)
- $MgSO_4$—0.0081 M (0.9750 gm. per liter)

Solution B: $Ca(NO_3)_2$—0.0101 M (1.6573 gm. per liter)

Enough of these salts to make 100 liters of culture solution were dissolved and made up to 2 liters, the $Ca(NO_3)_2$ being made up in a separate 2-liter portion in order to prevent precipitation of insoluble calcium salts in the highly concentrated solution. The mixture of these two solutions was designated solution A B, and 7.5 cc. of each of these solutions were added to 1500 cc. of distilled water for the initial dose of nutrient solution. This quantity of nutrient solution was applied to the jars designated in the outline of the scheme of the experiment at the time of planting (March 1). In addition, 0.01 gm. of $FeCl_3$ was added to each culture one week after sowing. On April 4 each A B culture received a second dose of 7.5 cc. of this normal nutrient solution. All cultures receiving only A B solutions will be referred to hereafter as "normal."

Solutions lacking in P, N, and K were also made. The amount of salts indicated in the respective tables were dissolved and made up to 2000 cc. with distilled water; 75 cc. of these solutions made up to 1500 cc. were used as initial doses. Similarly, solutions were made up in which the P, N, and K were supplied in one-fourth the concentration of that found in solution A B.

Solution C
(lacking in phosphorus)

3.437 gm. KNO_3
8.8561 gm. KCl
23.855 gm. $Ca(NO_3)_2$, $4H_2O$
19.789 gm. $MgSO_4$, $7H_2O$

Solution D
(phosphorus in one-fourth concentration)

3.437 gm. KNO_3
3.873 gm. KH_2PO_4
6.642 gm. KCl
23.855 gm. $Ca(NO_3)_2$, $4H_2O$
19.789 gm. $MgSO_4$, $7H_2O$

Solution E
(lacking nitrogen)

2.723 gm. KCl
14.6923 gm. KH_2PO_4
12.353 gm. $CaCl_2$, $2H_2O$
19.789 gm. $MgSO_4$, $7H_2O$

Solution F
(nitrogen in one-fourth concentration)

0.8592 gm. KNO_3
2.0422 gm. KCl
14.6923 gm. KH_2PO_4
19.7890 gm. $MgSO_4$, $7H_2O$
4.1432 gm. $Ca(NO_3)_2$, $4H_2O$
9.264 gm. $CaCl_2$, $2H_2O$

Solution G
(lacking potassium)

2.8894 gm. $NaNO_3$
17.0692 gm. Na_2HPO_4, $12H_2O$
23.855 gm. $Ca(NO_3)_2$, $4H_2O$
19.789 gm. $MgSO_4$, $7H_2O$

Solution H
(potassium in one-fourth concentration)

0.8592 gm. KNO_3
2.1671 gm. $NaNO_3$
3.873 gm. KH_2PO_4
12.8019 gm. Na_2HPO_4, $12H_2O$
19.789 gm. $MgSO_4$, $7H_2O$
23.8558 gm. $Ca(NO_3)_2$, $4H_2O$

To certain of the A B cultures extra doses of N, K, and P, alone, and in all possible combinations, were added in the amounts and at the times indicated in the schematic outline. These extra doses were supplied in the form of solutions of $NaNO_3$, KCl, and Na_2HPO_4, $12H_2O$ respectively. All cultures were run in triplicate. Certain of the replicates in each set of triplicates received a modified supplementary treatment, as indicated in table II, the letters N, K, and P indicating $NaNO_3$, KCl, and Na_2HPO_4, $12H_2O$ respectively.

Oderbrucker barley (Wisconsin No. 5) was seeded March 1. About 30 seeds were sown per culture, the cultures being thinned to 25 plants per culture. This heavy seeding was purposely chosen in order to prevent tillering, so that the course of development of a plant with a single culm could be followed.

TEMPERATURE AND HUMIDITY CONTROL.—The temperature of the greenhouses was controlled by means of automatic thermoregulators. The lower temperature selected was 15° C., the higher temperature 20° C. The degree of control obtained is shown in

TABLE II

OUTLINE OF GREENHOUSE EXPERIMENTS

Jar no.*	General treatment	Supplementary treatment
1, 2, 3 64, 65, 66	Distilled water only	.....
4, 5 68, 69	Solution C	.....
6 67	Solution C	2 gm. P added April 27
7, 8, 9... 70, 71, 72	Solution D	1 gm. N added April 26
10, 11, 12 73, 74, 75	Solution A B	1 gm. N added April 26
13, 14, 15 76, 77, 78	Solution A B+1 gm. P	Second dose of 1 gm. of P added March 30
16, 17 80, 81	Solution E	.....
18 71	Solution E	4 gm. N added April 27
19, 21 82, 84	Solution F	.....
20 83	Solution F	4 gm. N added April 27
22, 23 85, 86	Solution A B+1 gm. N	Second dose of 1 gm. of N on March 20; third dose of 2 gm. on April 26; fourth dose of 2 gm. on April 29
24 87	Solution A B+1 gm. N	Second dose of 1 gm. of N on March 30
25, 26, 27 88, 89, 90	Solution A B	1 gm. N added April 26
28, 30 91, 92	Solution G	1 gm. N added April 27
29 93	Solution G	1 gm. N added April 27; 2 gm. K added April 27
31, 32, 33 94, 95, 96	Solution H	1 gm. N added April 27
34, 35, 36 97, 98, 99	Solution A B	1 gm. N added April 27
37, 38, 39 100, 101, 102	Solution A B+1 gm. KCl	Second dose of 1 gm. K March 30; 1 gm. N April 26
40, 42 103, 104	Solution A B+1 gm. N, 1 gm. P	Second dose of 1 gm. N and 1 gm. P March 30; third dose of 2 gm. of each April 26; 2 gm. N only April 29
41 105	Solution A B+1 gm. N, 1 gm. P	Second dose of 1 gm. N and 1 gm. P March 30; 1 gm. N and 2 gm. P April 26
43, 45 106, 107	Solution A B+1 gm. N	Second dose of 1 gm. N March 30; third dose of 1 gm. N April 26
44 108	Solution A B+1 gm. N	Second dose of 1 gm. N April 26
46, 47, 48 109, 110, 111	Solution A B+1 gm. P, 1 gm. K	Second dose of 1 gm. of P and 1 gm. K March 30; 1 gm. N April 26
49, 50	Solution A B+1 gm. N	Second dose of 1 gm. N and 1 gm. K March 30; third dose of 2 gm. of each April 26; 2 gm. more of N April 29

TABLE II—*Continued*

Jar no.	General treatment	Supplementary treatment
51, 114	Solution A B+1 gm. N, 1 gm. K	Second dose of 1 gm. N and 1 gm. K March 30; 1 gm. N and 2 gm. K April 26
52, 53, 54, 115, 116, 117	Solution A B+1 gm. P	Second dose of 1 gm. P March 30; 1 gm. N April 26
55, 56, 119, 120	Solution A B+1 gm. N, 1 gm. K, 1 gm. P	Second dose of 1 gm. each of N, K, and P March 30; of 2 gm. each April 26; 2 gm. N April 29
57, 118	Solution A B+1 gm. N, 1 gm. K, 1 gm. P	Second dose of 1 gm. each of N, K, and P March 30; 1 gm. N and 2 gm. each of K and P April 26
58, 59, 122, 123	Solution A B+1 gm. N, 1 gm. P	Second dose of 1 gm. each of N and P March 30; 2 gm. more of each April 26; 2 gm. of N April 29
60, 121	Solution A B+1 gm. N, 1 gm. P	Second dose of 1 gm. each of N and P March 30; 1 gm. N and 2 gm. P April 26
61, 62, 124, 125	Solution A B+2 gm. P, 1 gm. N	Second dose of 2 gm. P and 1 gm. N March 30; 4 gm. P and 2 gm. N April 26; 2 gm. N April 29
63, 126	Solution A B+2 gm. P, 1 gm. N	Second dose of 2 gm. P and 1 gm. N March 30; 4 gm. P and 1 gm. N April 26

* Jars nos. 1–63 inclusive kept in warm greenhouse; jars nos. 64–126 inclusive kept in cool greenhouse

the thermograph records obtained in the two houses (figs. 1, 2). It will be noted that there was a fairly satisfactory degree of control up to about the middle of April, at which time (April 19) the samples for chemical analyses were taken. The principal fluctuations came at about noon; a considerable temperature difference always existed.

The degree of humidity was not under a complete control as desired, the evaporation rate averaging somewhat higher in the warm house. It is possible that some of the differences noted in chemical composition are due to the higher evaporating power of the air in the warm house. This higher evaporation rate was, of course, a function of the higher temperature.

Observations on growth of barley cultures

During the first two weeks of growth the plants in the warm house, which were several inches high before the plants in the cool house had come up, maintained a more rapid growth rate. The first leaves of all of the plants in the warm house, except those receiving little or no nitrogen, tended to lop over. The low

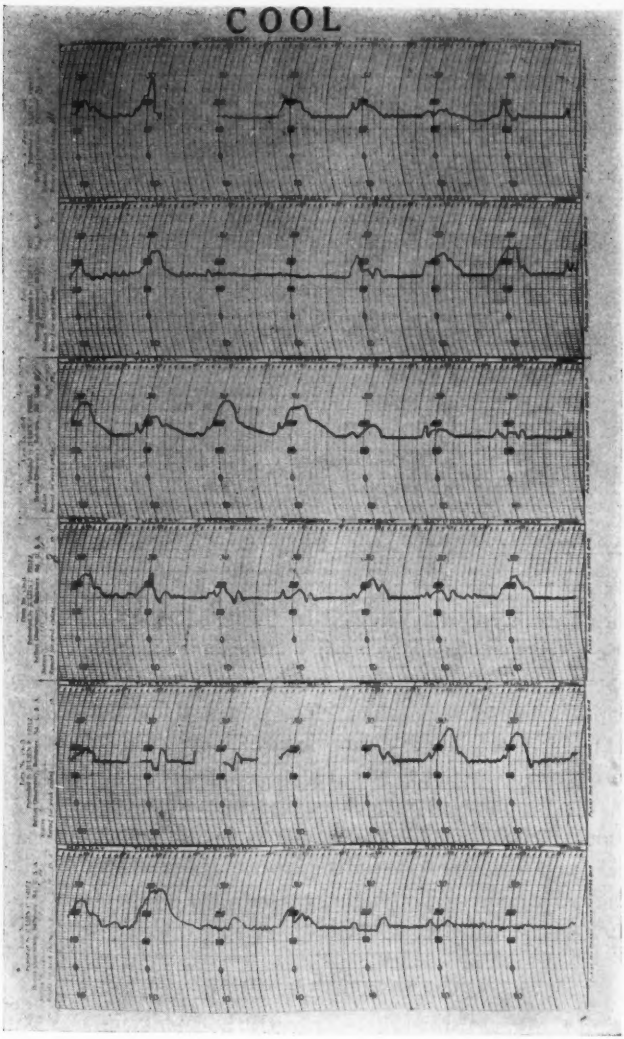

FIG. 1.—Thermograph records showing air temperature in cool house from planting to time of sampling for chemical analysis

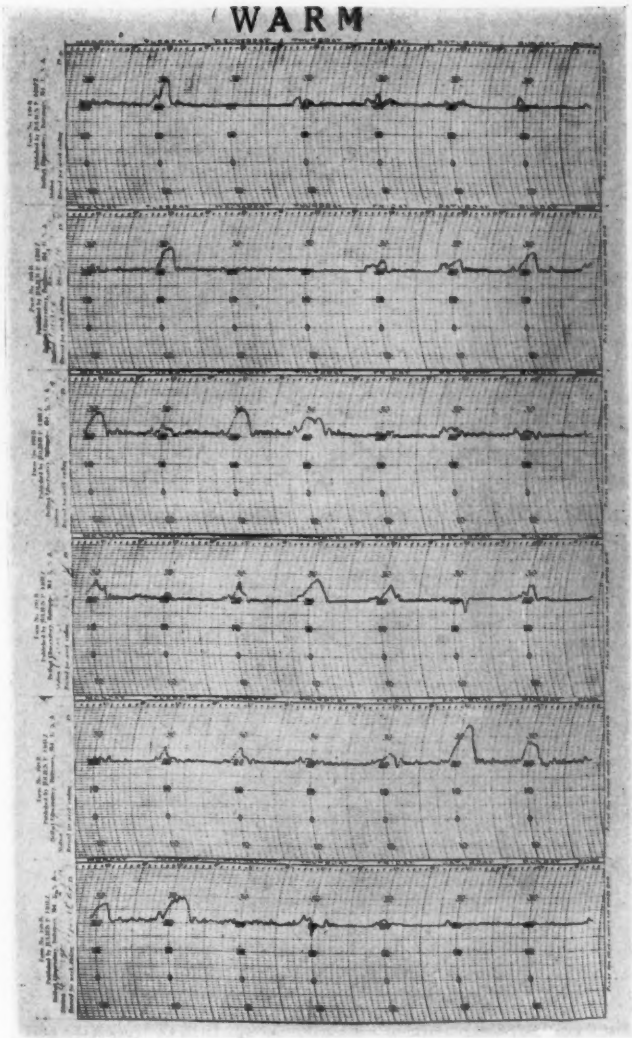

FIG. 2.—Thermograph records showing air temperature in warm house from planting to time of sampling for chemical analysis

nitrogen leaves were in every case stiff and upright. By March 16 the "no phosphorus" series began to show the effect of the deficiency. The "no potassium" series in the warm house showed the

TABLE III

PROPORTION OF LEAVES (BLADE AND SHEATH) AND STEMS IN 100 PARTS OF TOTAL PLANTS, BASED ON GREEN WEIGHT

Culture no. and treatment	Leaves (blades and sheaths) Percentage	Stems Percentage
44. High N warm	92.95	7.05
24. High N warm	88.23	11.73
40. High P and N warm	96.87	3.23
108. High N cool	69.20	30.80
87. High N cool	65.68	34.32
104. High P and N cool	71.37	28.63

greatest lopping over on March 16. About April 1 the plants in the cool house began to outstrip the plants in the warm house in their growth rate, and in particular in their tendency to maintain an upright growth habit. The total amount of tissue formed at

FIG. 3.—Nitrogen series, cool house: note vigorous upright condition of no. 85 as compared with sprawling condition of no. 23 (fig. 4).

the higher temperature was about the same, but it was differently distributed, as will be apparent from the data given in table III.

By April 19 all plants in the cool house had outstripped those in the warm house. The most striking difference between the two

houses was the sprawling condition of the high nitrogen cultures in the warm house, in contrast with their upright condition in the cool house. Figs. 3–15, taken April 24, show the condition of the barley on that date.

FIG. 4.—Nitrogen series, warm house

FIG. 5.—Phosphorus series, cool house: N and K treatment of nos. 68, 71, 75, and 78 "normal" (same as no. 89 in fig. 3).

Figs. 16–18, taken May 16, show the failure of the high nitrogen-high temperature plants to mature normally. Such shooting as was obtained at the higher temperature was due, in the opinion of the writer, to inability to control the moisture supply, because of very great fluctuation in the temperature as the spring season advanced. The writer believes that had it been possible to control absolutely

temperature and moisture supply, the high nitrogen-high temperature series could have been maintained in practically continuous vegetation without any tendency to reproduce. The reason for this belief was the failure of this series to produce any stem (culm) until the water supply fell below the normal previously maintained.

Chemical examination of tissues

In order to ascertain, if possible, the character of the internal processes that determine this very striking formative effect of the

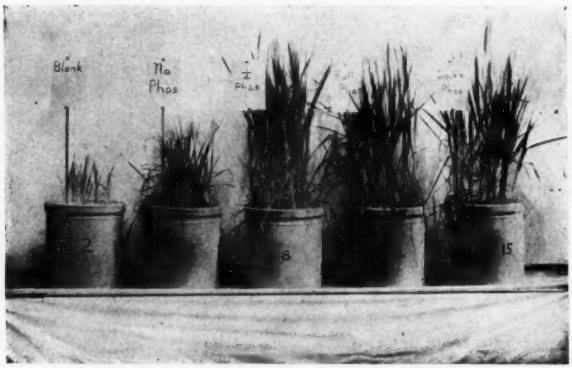

FIG. 6.—Phosphorus series, warm house: N and K treatment of nos. 6, 8, 12, and 15 "normal" (same as no. 25 in fig. 4).

higher temperature in the presence of high nitrogen supply, tissue analyses were made on the leaves (blades plus sheaths) from 100 gm. of total plants from cultures nos. 44, 24, 108, 87, and 104. The plants were selected so that the sample equaled 100 gm. Table III shows the very low percentage of stem material at the higher temperature. Since both leaf-blade and leaf-sheath are active organs in cereals, both were included. The second column in the table shows the green weight in grams of this leaf tissue. The first column in table VI shows the date and hour of taking these samples.

Methods of tissue analysis

The green samples were weighed and immediately preserved by adding enough ethyl alcohol to make a 75 per cent alcoholic solution, and then boiled to arrest enzymic activity. The preserved

Fig. 7.—Potassium series, cool house: N and P treatment of nos. 92, 94, and 100 same as no. 89 in fig. 3; note sprawling condition of "no potash" culture.

Fig. 8.—Potassium series, warm house: N and P treatment of nos. 29, 32, and 38 same as no. 25 in fig. 4.

material was then subjected to the method of tissue analysis devised by Waldemar Koch, and modified by F. C. Koch (**8**). The method used consisted essentially of 4 hours' extraction with hot ethyl alcohol in a continuous extractor, followed by 1 hour's

extraction with ether, then treatment of the finely ground material with hot water several times, after which the aqueous mixture was made up to a concentration of 75 per cent alcohol and filtered. The insoluble material was then subjected to further extraction with hot alcohol for 24 hours.

The combined extractions were evaporated to dryness on a steam bath, then repeatedly evaporated with absolute ethyl alcohol in order to remove water. The dry hard residue was then

FIG. 9.—Effect of heavy N fertilization: no. 12, normal N (warm house); no. 85, heavy N (cool house); no. 22, heavy N (warm house); no. 75, normal N (cool house).

extracted with anhydrous ether by grinding with a pestle with successive portions of fresh ether. The ethereal extracts were made up to 250 cc., and then divided into suitable aliquots for chemical and dry weight determinations (50 cc. portions). This extraction was designated as fraction 1 (F_1). The ether-insoluble residue was taken up in about 65 per cent alcohol and made up to a volume of 500 cc., 50 cc. portions being taken as aliquots for analysis and dry weight determinations. This was designated as fraction 2 (F_2). Moisture determinations were made on duplicate F_1 and F_2 aliquots

by evaporating almost to dryness on the steam bath and then taking down to constant weight in a vacuum desiccator.

TABLE IV

EFFECT OF TEMPERATURE UPON AMOUNT AND PERCENTAGE OF DRY MATTER AND WATER IN BARLEY LEAVES

Culture no. and treatment	Green weight (gm.)	Dry weight (gm.)	Weight of water (gm.)	Percentage of water	Percentage of dry matter
44. High N warm.....	92.95	12.67	80.28	86.36	13.64
24. High N warm.....	88.23	12.79	75.44	85.50	14.50
41. High P and N warm...	96.87	11.10	85.77	89.47	10.53
108. High N cool.....	69.20	10.92	58.28	84.21	15.79
87. High N cool.....	65.68	10.23	55.45	84.42	15.58
104. High P and N cool....	71.37	11.04	60.23	84.39	15.61

Material insoluble in ether, alcohol, and water was designated as fraction 3 (F_3). This entire fraction was dried to constant weight

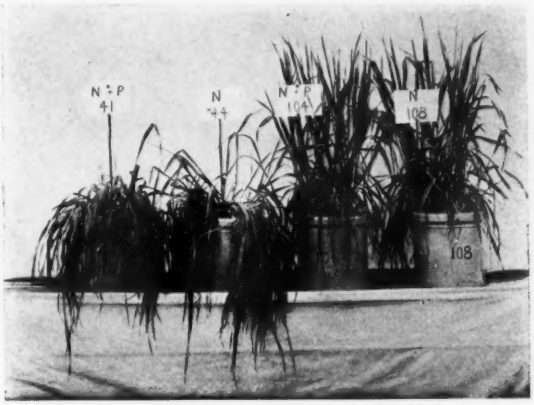

FIG. 10.—Influence of supplementary P fertilization on heavy N fertilization: all cultures received equal heavy doses of N in form of $NaNO_3$; cultures nos. 41 and 104 received equal dosage of extra P; nos. 41 and 44 grown in warm house; nos. 104 and 108 in cool house; P failed to counteract effects of N at higher temperature; chemical analyses made of leaves from this set of cultures.

at 100° C. in an electrically heated oven. Table IV gives the relative proportions of moisture and dry matter in the several samples analyzed.

Table V gives the distribution of the several fractions in the samples analyzed. Particular attention is directed to the fact that the temperature does not seem to have any important effect upon the proportion of lipins (F_1), except where extra phosphorus is present, in which case a high temperature led to an increase in the lipin material. The author regrets not being able to confirm this

FIG. 11.—Influence of supplementary K fertilization on heavy N fertilization: nos. 50 and 113 received equal heavy doses of N in form of $NaNO_3$; nos. 100 and 38 received only "normal" N; all 4 cultures received equal heavy doses of K in form of KCl; nos. 50 and 38 warm house; nos. 100 and 113 cool house; K failed to counteract effects of N at higher temperature.

interesting observation by means of further analyses. The proportion of fraction 2, which might quite properly be designated the metabolic fraction, averages about 10 per cent higher at the higher temperature. The proportion of fraction 3, or storage and skeleton fraction, averages nearly 8 per cent higher at the lower temperature.

F_1 was analyzed for total N and total P. F_2 was analyzed for total N (organic and ammoniacal only), total P, direct reducing

sugars, and for total sugars after mild hydrolysis. Samples 24 and 87 were also analyzed for inorganic phosphorus, using the POWICK-CHAPIN (10) method. F_3 was analyzed for total N, total P, N and P soluble and insoluble in 1 per cent NaOH, phosphoprotein phosphorus, polysaccharides, and cellulose, etc., by

TABLE V

EFFECT OF TEMPERATURE ON DISTRIBUTION OF EXTRACTIVES AND INSOLUBLE MATTER IN BARLEY LEAVES

Culture no. and treatment	Soluble in anhydrous ether (F_1) Percentage	Soluble in hot alcohol and water (F_2) Percentage	Insoluble in ether, alcohol, and water (F_3) Percentage
44. High N warm	8.699	33.017	58.284
24. High N warm	7.885	33.743	58.372
41. High P and N warm	10.433	32.613	56.954
108. High N cool	8.251	30.321	61.428
87. High N cool	8.663	27.823	63.514
104. High P and N cool	7.681	30.279	62.040

difference. The following list gives the methods employed. The details of the several methods are those recommended by KOCH (8) and MATHEWS (9).

Total nitrogen.....Arnold-Gunning method.
Total phosphorus.....Neuman-Pemberton method.
Direct reducing sugars...Bertrand volumetric method (glucose calculated from Munson-Walker tables in MATHEW'S *Physiological Chemistry*).
Total sugars.....Bertrand volumetric method applied to the products of mild hydrolysis with HCl.
Polysaccharides.....Bertrand volumetric method applied to the products of strong hydrolysis with HCl.
Phosphoprotein phosphorus.....Determination of the P precipitable by Mg mixture in an extract made by 48 hours' digestion with 1 per cent NaOH at 37–40° C.

The method for phosphoprotein phosphorus is based upon the discovery by PLIMMER and SCOTT that phosphoproteins can be separated from nucleoproteins through hydrolyzing the former with 1 per cent NaOH, the latter being unattacked by the dilute

alkali. The exact details of the method used on this material are as follows. Weighed samples of F_3 were placed in 300 cc. Erlenmeyer flasks, usually about 0.5 gm., and 1 per cent NaOH, free from phosphorus, was then added at the rate of 100 cc. of NaOH for each 1.0 gm. of substance. The flasks were stoppered and placed in an electric incubator at 37–40° C., where they were allowed

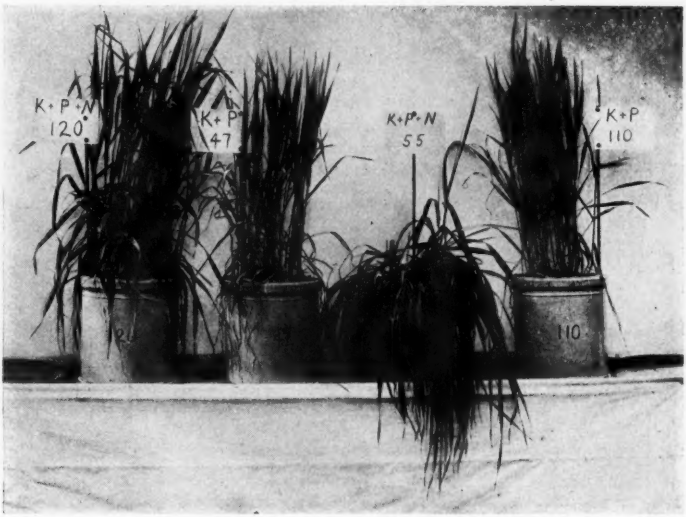

FIG. 12.—Influence of supplementary fertilization with both K and P on heavy N fertilization: no. 120, heavy N+extra K and P, cool house; no. 47, "normal" N+extra K and P, warm house; no. 55, heavy N+extra K and P, warm house; no. 110, "normal" N+extra K and P, cool house; note that "complete fertilizer" failed to counteract effects of heavy N at higher temperature; are not growth effects noted in no. 55 referable to stimulus received at time of germination?

to remain 48 hours. The flasks were shaken about 4 times each day. At the end of the digestion period the insoluble material was filtered off on ashless filter papers and carefully washed with lukewarm water. The combined filtrate and washings were then neutralized to litmus with acetic acid and the PO_4 ions precipitated with magnesia mixture in the presence of an excess of NH_4OH. This precipitation was conducted at a low temperature, the solutions

being allowed to stand in the ice box for 24 hours. At the end of the 24 hour period the magnesium ammonium phosphate was filtered off, washed with 2.5 per cent cold ammonia water, dissolved

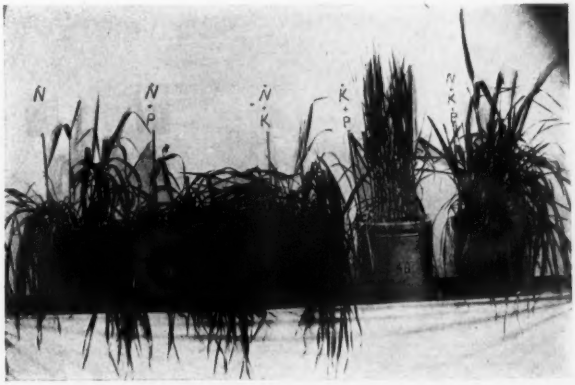

FIG. 13.—Influence of variation in fertilization in warm house: N, K, and P indicate that fertilizer dosage is in excess of "normal" A B solution; contrast with results shown in fig. 14, where fertilizer treatment is identical but temperature lower.

FIG. 14.—Influence of variation of fertilization in cool house: contrast with fig. 13

in dilute nitric acid, and the phosphorus then precipitated by means of the molybdate solution. Final determination of the phosphorus was made by means of the Pemberton alkalimetric method.

In order to determine whether or not the material thus extracted by 1 per cent NaOH contained any forms of P not precipitated by magnesia mixture, the phosphorus was determined in the insoluble residue. Similarly total N determinations were made in the insoluble residue from another set of determinations. The difference

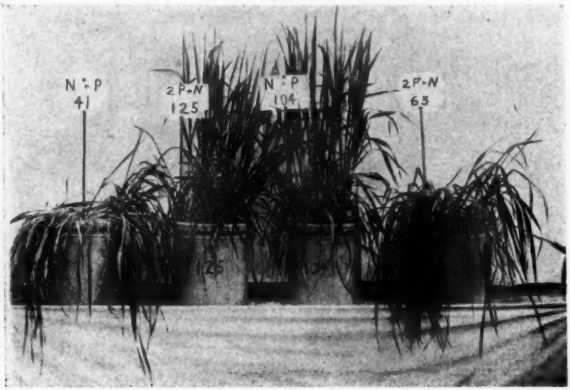

FIG. 15.—Influence of extra heavy supplementary P fertilization on heavy N fertilization: no. 41, heavy N and heavy P (warm house); no. 125, heavy N and extra heavy P (cool house); no. 104, heavy N and heavy P (cool house); no. 63, heavy N and extra heavy P (warm house).

between total P (or N) soluble in 1 per cent NaOH gave the P (or N) present in the NaOH extract.

TABLE VI

EFFECT OF TEMPERATURE ON ACCUMULATION OF SOLUBLE CARBOHYDRATES IN BARLEY LEAVES (RESULTS OF ANALYSIS OF F_2)

CULTURE NO., TREATMENT, AND TIME OF SAMPLING	DIRECT REDUCING SUGARS (AS GLUCOSE)		TOTAL SUGARS AFTER MILD HYDROLYSIS (AS GLUCOSE)		PERCENTAGE CONCENTRATION OF TOTAL SUGARS (AS GLUCOSE) IN TOTAL WATER IN TISSUE
	Percentage F_2	Percentage total leaf	Percentage F_2	Percentage total leaf	
44. High N 4 P.M., April 24, 1918 warm.....	15.09	5.27	29.41	9.71	0.15252
24. High N 9 A.M., April 25, 1918 warm.....	19.83	6.69	26.84	9.05	0.15342
41. High P and N 3 P.M., April 25, 1918 warm..	10.62	3.46	18.55	5.05	0.06535
108. High N 6 P.M., April 24, 1918 cool.....	20.20	6.11	45.79	13.88	0.26007
87. High N 10 A.M., April 24, 1918 cool.....	23.17	6.44	36.99	10.29	0.18985
104. High P and N 5 P.M., April 24, 1918 cool...	25.08	7.59	39.90	12.08	0.22142

Tables VI–XII contain the results of the different determinations. Table XIII gives the proportion of skeletal material in

F_3, calculated as the difference between the total amount of the fraction, and the sum of the protein and starch in the fraction.

TABLE VII

EFFECT OF TEMPERATURE ON ACCUMULATION OF POLYSACCHARIDES IN BARLEY LEAVES

Culture no. and treatment	Percentage of F_3	Percentage of total leaf
44. High N warm	21.16	12.33
24. High N warm	21.64	12.63
41. High P and N warm	20.43	11.63
108. High N cool	25.51	15.67
87. High N cool	23.60	14.99
104. High P and N cool	24.14	14.97

TABLE VIII

EFFECT OF TEMPERATURE ON AMOUNT OF NITROGEN, PHOSPHORUS, PROTEIN, AND PHOSPHOPROTEIN PHOSPHORUS IN BARLEY LEAVES (PERCENTAGE OF TOTAL DRY WEIGHT)

Culture no. and treatment	Total N	Total P	Total protein (Percentage N in F_3×6.25)	Phosphoprotein phosphorus
44. High N warm	2.9779	0.6944	12.965	0.1167
24. High N warm	2.7007	0.6789	11.277	0.1411
41. High P and N warm	3.4661	0.7988	14.425	0.0795
108. High N cool	2.5610	0.5479	12.865	0.0543
87. High N cool	2.6065	0.5711	13.159	0.0832
104. High P and N cool	2.5945	0.6532	12.008	0.0996

TABLE IX

EFFECT OF TEMPERATURE ON AMOUNT AND DISTRIBUTION OF NITROGEN IN BARLEY LEAVES

Culture no. and treatment	F_1 soluble in anhydrous ether			F_2 soluble in hot alcohol and water			F_3 insoluble in alcohol, ether, or water		
	Percentage fraction	Percentage total leaf	Percentage total N in leaf	Percentage fraction	Percentage total leaf	Percentage total N in leaf	Percentage fraction	Percentage total leaf	Percentage total N in leaf
44. High N warm	1.4993	0.1304	4.38	2.3420	0.7732	25.96	3.559	2.0743	69.66
24. High N warm	1.3081	0.1021	3.78	2.1312	0.7191	26.62	3.237	1.8795	69.60
41. High P and N warm	2.6700	0.2785	8.03	2.6970	0.8795	25.377	4.053	2.3081	66.60
108. High N cool	1.4142	0.1167	4.55	1.2730	0.3859	15.07	3.351	2.0584	80.38
87. High N cool	1.2520	0.1084	4.16	1.4110	0.3025	15.06	3.315	2.1054	80.78
104. High P and N cool	1.3205	0.1014	3.91	1.4130	0.4278	16.49	3.329	2.0653	79.60

TABLE X

EFFECT OF TEMPERATURE ON AMOUNT AND DISTRIBUTION OF PHOSPHORUS IN BARLEY LEAVES

CULTURE NO. AND TREATMENT	F_1 SOLUBLE IN ANHYDROUS ETHER			F_2 SOLUBLE IN HOT ALCOHOL AND WATER			F_3 INSOLUBLE IN ALCOHOL, ETHER, AND WATER		
	Percentage fraction	Percentage total leaf	Percentage total P in leaf	Percentage fraction	Percentage total leaf	Percentage total P in leaf	Percentage fraction	Percentage total leaf	Percentage total P in leaf
44. High N warm	0.7369	0.0641	9.23	0.8683	0.2866	41.28	0.5898	0.3437	49.49
24. High N warm	0.6847	0.0539	7.93	0.8211	0.2770	40.80	0.5960	0.3478	51.27
41. High P and N warm	0.7857	0.0819	10.26	1.3394	0.4368	54.69	0.4918	0.2800	35.05
108. High N cool	0.7739	0.0638	11.65	0.5352	0.1622	29.62	0.5239	0.3218	58.73
87. High N cool	0.7247	0.0627	10.99	0.5096	0.1417	24.80	0.5614	0.3565	64.21
104. High P and N cool	0.7975	0.0612	9.37	0.9602	0.2907	44.50	0.4856	0.3012	46.13

TABLE XI

EFFECT OF TEMPERATURE UPON SOLUBILITY OF F_3 NITROGEN OF BARLEY LEAVES IN 1 PER CENT NaOH (MATERIAL DIGESTED WITH 1 PER CENT NaOH FOR 48 HOURS AT 37–40° C.)

CULTURE NO. AND TREATMENT	SOLUBLE NITROGEN			INSOLUBLE NITROGEN		
	Percentage fraction	Percentage total leaf	Percentage total N in leaf	Percentage fraction	Percentage total leaf	Percentage total N in leaf
44. High N warm	0.910	0.5303	17.81	2.649	1.5440	51.85
24. High N warm	0.8570	0.4902	18.17	2.380	1.3892	51.43
41. High P and N warm	1.126	0.6411	18.50	2.927	1.6670	48.10
108. High N cool	0.873	0.5361	20.93	2.478	1.5221	59.43
87. High N cool	1.052	0.6681	26.03	2.263	1.4373	54.75
104. High P and N cool	0.923	0.5726	22.07	2.406	1.4926	57.53

TABLE XII

EFFECT OF TEMPERATURE UPON SOLUBILITY OF F_3 PHOSPHORUS OF BARLEY LEAVES IN 1 PER CENT NaOH (MATERIAL DIGESTED WITH 1 PER CENT NaOH FOR 48 HOURS AT 37–40° C.)

CULTURE NO. AND TREATMENT	SOLUBLE PHOSPHORUS (BY DIFFERENCE)			INSOLUBLE PHOSPHORUS		
	Percentage fraction	Percentage total leaf	Percentage total P in leaf	Percentage fraction	Percentage total leaf	Percentage total P in leaf
44. High N warm	0.2292	0.1335	19.22	0.3606	0.2102	30.27
24. High N warm	0.1994	0.1163	17.13	0.3966	0.2315	34.10
41. High N and P warm	0.1959	0.1115	13.96	0.2959*	0.1685*	21.09
108. High N cool	0.1132	0.0696	12.70	0.4107	0.2522	46.03
87. High N cool	0.1807	0.1148	20.10	0.3807	0.2417	42.32
104. High N and P cool	0.1666	0.1039	15.91	0.3190†	0.1979†	30.30

* Poor duplicates.

† One analysis only, duplicate lost.

TABLE XIII

EFFECT OF TEMPERATURE UPON AMOUNT OF CELL WALL MATERIAL, ETC. $F_3-[(N \text{ IN } F_3 \times 6.25)+(\text{STARCH IN } F_3)]$; EXPRESSED AS PERCENTAGE OF TOTAL DRY WEIGHT OF LEAF

Culture no. and treatment	Cell wall material, etc.	Ratio of supporting tissue (cell walls, etc.) to all other plant substances, including water
44. High N warm	32.99	0.0470
24. High N warm	34.47	0.0525
41. High P and N warm	30.90	0.0367
Average warm	32.78	0.0454
108. High N cool	32.89	0.0539
87. High N cool	35.36	0.0581
104. High P and N cool	34.16	0.0558
Average cool	34.13	0.0559

TABLE XIV

EFFECT OF TEMPERATURE ON DISTRIBUTION OF PHOSPHORUS; SUMMARY TABLE

Material	No. 24, high N, warm		No. 87, high N, cool	
	Percentage total leaf	Percentage total P	Percentage total leaf	Percentage total P
Lipoid P, F_1	0.0539	7.94	0.0627	10.99
Phosphate P, F_2	0.2105	31.01	0.0714	12.80
Organic P, F_2	0.0665	9.80	0.0703	12.31
Phosphoprotein P, F_3	0.1411	20.80	0.0832	18.38
Nucleoprotein P, F_3	0.2067	30.45	0.2833	49.62
Total P	0.6787		0.5709	

Results of chemical analysis

LIPIN FRACTION (F_1).—The results given in table V indicate that the temperature has very little effect upon the amount of lipins, except in the case of a high phosphorus supply, where the percentage of lipins is decidedly higher. This fact is possibly correlated with the higher percentage of phospho-lipin phosphorus in the entire leaf, as shown in the third column of table X, and the higher percentage of lipin N as shown in the third column of table IX. Since the proportion of lipin P is practically the same for both temperatures in the case of the high nitrogen series, these data lead to the conclusion that the lipin fraction is not an important growth determinant. The writer recognizes the desirability of more data.

ALCOHOL-WATER SOLUBLE FRACTION (F_2).—Table V shows a distinctly higher average percentage of these extractives at the higher temperature, although the order of difference is not large. When, however, the composition of this fraction is examined certain striking differences are noted. The high temperature leaves contain a much lower percentage of both total and reducing sugars (table VI) and a lower percentage of polysaccharides (table VII). The high temperature leaves contain about twice as much nitrogen (as determined by the unmodified Arnold-Gunning process) as do the low temperature leaves (table IX). In other words, the amount of active metabolic nitrogen, such as amino acids, polypeptides, and simpler water soluble proteins, is much higher at the higher temperature. The amount of nitric N is also higher at the higher temperature, as was indicated when the modified Arnold-Gunning process was used. The results of the nitric N determinations are not reported in this paper. The high temperature leaves also contain nearly twice the percentage of alcohol-water soluble phosphorus. Duplicate determinations on one set of samples (nos. 24 and 87) indicated that this difference was very largely due to the much higher percentage of inorganic phosphorus at the higher temperature. These results are appended, although it is recognized that more data are needed before any sweeping generalizations can be made. The Powick-Chapin method was used in this determination.

FIG. 16.—Influence of temperature on maturation (photographed May 16); no. 12, "normal" fertilization (warm house); no. 74, "normal" fertilization (cool house).

	TOTAL P		INORGANIC P	
	Percentage of fraction	Percentage of entire leaf	Percentage of fraction	Percentage of entire leaf
No. 24	0.8211	0.2770	0.6240	0.2105
No. 87	0.5096	0.1417	0.2567	0.0714

FRACTION 3.—The higher amount of polysaccharides at the lower temperatures has been noted. Table V shows that the leaves grown at the lower temperature contain a distinctly higher average percentage of this fraction, although the order of difference is not large. Tables IX and X show that there is no important

FIG. 17

FIG. 18

FIGS. 17–18.—Fig. 17, influence of heavy N and heavy K on maturation (photographed May 16): no. 49, heavy N+heavy K (warm house); no. 112, heavy N+heavy K (cool house); comparison with other sets not shown indicate that K has no effect in causing difference; contrast heavy N cultures with normal N cultures of fig. 16; fig. 18, influence of heavy N and extra heavy P on maturation (photographed May 16): no. 63, heavy N+extra heavy P (warm house); no. 126, heavy N+extra heavy P (cool house); contrast with nos. 63 and 125 (same treatment) in fig. 15.

difference in the percentage of either N or P at the different temperatures. The amount of phosphoprotein phosphorus seems to run somewhat lower at the lower temperature (table VIII).

In five out of six cases (cf. column 3, table XII, with column 5, table VII) the amount of phosphorus in the NaOH extract exceeded the phosphorus precipitable from that extract by 1 per cent NaOH, indicating that either some organic phosphorus compounds had

been dissolved by the NaOH but had not been hydrolyzed, or that the magnesia mixture failed to give quantitative precipitations of the PO_4 ions under the conditions of the experiment.

Table IX reports a study of the solubility of the F_3 nitrogen in 1 percentage NaOH. The results are inconclusive, but are reported for the sake of completeness.

The calculations reported in table XIII are self-explanatory. It will be noted that the average proportions of framework material are considerably higher at the lower temperature. Microchemical examination of median cross-sections of the leaves and of the culms showed a greater degree of lignification of the xylem bundles at the lower temperature, a fact of added significance. Lignification of the vessels in the culm adds greatly to the strength of the stem. Referring to the enormous differences in growth habit as shown in the figures, we may conclude that the upright habit at the lower temperature is due to: (1) a greater proportion of culm to leaf; (2) a greater proportion of skeletal material in the leaf; (3) a greater degree of lignification of conductive tissues in both leaf and culm. These obvious anatomical facts, however, are but the expression of a difference in metabolic equilibria, especially the nitrogen-carbohydrate ratio.

Discussion

The experiments reported in this paper, as well as the results of earlier investigators, reopen the question as to just what is meant by an optimum germination temperature. The classical investigations of HABERLANDT on germination temperature place the optimum at the temperature which most quickly permits the emergence of the radicle and plumule; in fact, practically all germination studies have been based upon this as the optimum. These optimum temperatures, at least for the cereals, are evidently too high to insure a future normal development. The writer believes that the course of development is to a large extent predetermined at a very early stage in the development of the plant by the chemical equilibria within the seedling, especially the nitrogen-carbohydrate ratio. These equilibria within the plant, like chemical reactions *in vitro*, are conditioned by the temperature and concentrations of the reacting substances. It seems likely that a high temperature

and a high nitrogen supply at an early stage in the development of the barley plant so shifts the equilibrium toward excessive vegetation as to prevent the normal tendency toward reproduction. Some other factor must be altered, therefore, as, for example, the water supply, if such plants are to be thrown into reproduction.

An investigation of the nitrogen-carbohydrate ratio at a different stage in the development of seeds and seedlings furnished with varying concentrations of nitrogenous compounds will probably throw considerable light upon these questions.

Conclusions

1. The excessive leaf production in the high temperature barley is caused by the high concentration of nitrates in the nutrient supplied.

2. Nitrate nitrogen in the nutrient begins to affect the subsequent course of development at high temperatures at the time of germination, or at least at a very early stage in the development of the plant. The tendency to excessive vegetation thus inaugurated cannot be counteracted by the addition of phosphorus or potassium salts.

3. The effect of the nutrient supply is reflected in the composition of the active organ, the leaf. The following equations represent the main facts revealed by chemical analysis of the leaf:

High heat supply + high nitrogen supply in nutrient solution = high soluble nitrogen in leaf + low soluble carbohydrate = excessive vegetation and little culm formation.

Low heat supply + high nitrogen supply in nutrient solution = low soluble nitrogen in leaf + high soluble carbohydrate = normal vegetation and normal culm formation.

The writer gratefully acknowledges his indebtedness to Professor WILLIAM CROCKER for helpful advice and criticisms; to Professor F. C. KOCH for valuable advice and laboratory facilities; and to the Department of Zoölogy of the University of Chicago for facilities afforded in their greenhouses.

AGRICULTURAL COLLEGE
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PHYSIOLOGICAL STUDY OF MAPLE SEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 260

H. A. JONES

(WITH TWO FIGURES)

Introduction

The appearance of two taxonomic species within the same genus is not always a criterion of similar physiological or ecological behavior. The seeds of two closely related species, as those of the sugar and river maples (*Acer saccharum* Marsh. and *A. saccharinum* L.), show a striking contrast in season of maturity, reaction to external conditions, chemical composition, and in their physiological behavior in general. The sugar maple matures its seeds in the fall, and these must pass through a well defined period of after-ripening before germination can take place. The storage substances are mainly protein and fat, with a small amount of carbohydrate present. On the other hand, the river maple ripens its seeds in the spring. The seeds germinate almost immediately upon a moist substratum, but if allowed to desiccate for some time under ordinary atmospheric conditions they soon lose their power of germination. A very small percentage of fat and protein is present, starch being the chief storage product.

It is a matter of common observation that many mature seeds and spores soon lose their power to germinate when subjected for varying periods to atmospheric desiccation. In a great many tropical seeds death follows atmospheric drying. In our own region the seeds of the willow and cottonwood are usually cited as the classic examples of death due to desiccation shortly after seed fall. The cottonwood gives low percentage of germination and low seedling vigor after two weeks of desiccation in laboratory air, while after three weeks seeds fail to germinate when placed in the most favorable germinative conditions. Cottonwood seeds, however, are in a high state of metabolic activity when first shed.

At 30° C. on moist filter paper the fresh seeds will usually give 100 per cent germination within 24 hours. The hypocotyls will attain a length of 8–9 mm., and the cotyledons will be entirely spread. SCHRÖDER (**23**) states that seeds of *Caltha palustris* failed to germinate after 11 weeks of storage over sulphuric acid and after 20 weeks of storage in the ordinary atmosphere. DELAVAN (**8**), working with the oaks and hickories, concludes that a cold even temperature, although the atmosphere be moist, is better than warm dry storage of seed. Seeds of *Oxalis*, elm, river maple, hornbeam, birch, beech, chestnut, and probably many others have their germinative power lowered or lost entirely by varying periods of desiccation.

Heretofore no work has been done on seeds, sensitive to drying, regarding the exact or approximate water content at the time of death. Furthermore, it has never been demonstrated whether loss of viability is due in part to temperature or entirely to desiccation effects.

Investigation

RIVER MAPLE (*Acer saccharinum* L.)

In the Chicago region *Acer saccharinum* matures its seeds the latter part of May or early in June, varying with the season. At the time of fall the seeds contain approximately 58 per cent of water, being almost fully imbibed. The seeds soon germinate if they lodge upon a moist substratum, but if they are subjected to desiccation there is an immediate reduction of the moisture content, and their viability is lost long before an air-dry condition is attained. The seeds of the river maple were chosen for this study because they are large, making it possible to obtain material readily in sufficient quantities for chemical analysis. The period of time between maturing and loss of viability is of moderate duration, permitting a study of internal changes accompanying desiccation; also seeds are abundant and easily collected. In all cases where reference is made to the maple fruit the seed plus the ovary wall is taken into consideration. Seed refers to the embryo plus the integuments. In all storage conditions the entire maple fruit was used; this holds for both the river and sugar maple. The criterion

for the beginning of germination is the protrusion of the tip of the hypocotyl through the integuments.

Water and temperature relations

Fruits were collected at time of shedding and stored at various constant temperatures from 0 to 40° C. At 25° C. and above fruits were stored in open wire baskets. At 20° C. and below they were stored in loosely covered cans which contained a considerable quantity of calcium oxide. The lime facilitated drying at the lower temperatures, besides preventing the accumulation of an excess carbon dioxide pressure about the seeds. By August 26, 1918, all seeds desiccated at 0–40° C. had lost their

TABLE I

LIFE DURATION OF SEEDS STORED AT VARIOUS DRYING TEMPERATURES

Storage temperature	Life duration*
35° C.	6 days
30	8
25	22
20	20
10	49
0	92

* At 25° C. the humidity of the air was considerably higher, and drying somewhat slower than at 20° C., accounting for increased life duration.

ability to germinate. In all cases seeds were considered to have lost their viability when 80 per cent failed to germinate when placed on moist filter paper at 30° C., all seeds having either germinated or decayed. From 0 to 35° C. the seeds lost their viability when the water content was reduced to 30–34 per cent. So far as could be determined, the various temperatures from 0 to 35° C. for desiccation do not appear to raise or lower the critical point of water content. At 40° C. death does not seem to be due to desiccation. Seeds turn black in a short time, killing apparently being due to the destructive action of this high temperature. One apparent effect of increasing temperatures (0–35° C.) is the shortening of the desiccation period, no change being evident in

the percentage of water at several temperatures at the time of loss of viability.

Seeds have a high metabolic activity at time of fall. Where viability and vigor are so closely allied with high water content, it is logical to suppose that the initial vigor can be retained for some time by holding the water percentage at the initial content, and by lowering the metabolic activity. Seeds at maturity and for some time thereafter give off considerable amounts of CO_2. For a number of samples at time of fall the yield of CO_2 was estimated as approximately 7 mg. per gram of dry weight per 24 hours at 25° C. If we consider 7 mg. as the amount of CO_2 respired in 24 hours at 25° C., the seeds would soon exhaust their store of food if the initial activity were maintained. The carbohydrate present would be entirely exhausted and the seeds die of starvation within approximately 120 days if this initial intense respiratory activity were maintained. At this rate it would be impossible to hold seeds just below the point of saturation at the higher temperature for any great length of time. Seeds, however, can be held for some time stored over water at low temperatures. Seeds harvested in the spring of 1917 were stored over water in desiccators at 10° C., and continued to give 95–100 per cent germination until November 1917. There was, however, an abnormal development of the hypocotyl during the latter part of the storage period at 10° C. No alkali was placed in the desiccators to prevent CO_2 accumulation, so it is impossible to say just what part was played by the carbon dioxide in the preservation of the seeds at this temperature. In the spring of 1918 seeds were stored over water in a large desiccator at 0° C. A bottle of strong alkali was also placed in the desiccator to prevent accumulation of a CO_2 blanket. These seeds were discarded after 102 days' storage, and at this time seeds were giving 100 per cent germination. They had retained their initial vigor and appeared to be normal in every respect. Perhaps many other seeds of this general behavior would retain their viability and vigor for considerable periods when placed in similar storage conditions. Seeds can be kept for a considerable period at temperatures just below the freezing point. After 50 days seeds stored at −5° C. gave good germination. At this low temperature care

must be taken that water does not come into contact with the outer walls of the fruit or integuments, as ice formed on the latter appears to inoculate the subcooled tissue below, and freezing to death results.

Respiration

Respiration was determined on newly collected seeds, on seeds desiccated at 25° C., and on germinating seeds. Determinations were made on the desiccating seeds every second day until viability was lost, and for several weeks thereafter. All respiration experiments were conducted at 25° C., as this temperature was thought to correspond very closely with the average temperature to which the seeds would be subjected under natural conditions. The method of determining the carbon dioxide given off was that described by GRAFE (12), with slight modifications. In general the method consists in pulling carbon dioxide free air over the respiring material through a column of barium hydroxide. The barium hydroxide solution is held by a Reiset tube. The air is drawn through slowly and uniformly. This is accomplished best by the air replacing water which is slowly siphoned out of a large demijohn by means of a capillary tube. At the end of a determination the barium carbonate was allowed to settle and an aliquot part (25 cc.) of the 100 cc. of barium hydroxide was pipetted off and titrated with N/20 oxalic acid. Phenolphthalein was the indicator used.

If the intensity of respiration may be used as a criterion of metabolic activity, then the seeds of the river maple at time of fall are in high state of metabolism. In the desiccating seeds there is a fall the first few days in respiratory activity, and then a gradual rise until a maximum is reached. This maximum is retained for several days, then there is a gradual decline, until only a trace of carbon dioxide is given off. This secondary rise in respiratory intensity may accompany increased starch hydrolysis. It will be seen later that accompanying desiccation there is a great increase in sucrose, due to starch hydrolysis. The later fall in respiratory activity is probably caused by a deficiency of water. The greatest respiratory activity was obtained on the desiccating seeds with a water content of approximately 44 per cent. There is no marked

degeneration of the respiratory enzymes during this fall, because when dead seeds are placed in germinative conditions the respiration again mounts to a high value, giving off 8.84 mg. of carbon dioxide per gram of dry weight in 24 hours. It is not known, however, just what percentage of the carbon dioxide given off in the latter case was due to bacterial action. HAAS (13) found that the marine alga *Laminaria*, in the presence of certain reagents, respired more rapidly after death than in the living condition. MAIGE and NICOLAS (17) have done considerable work on respiration in correlation with the state of turgidity of certain plant organs,

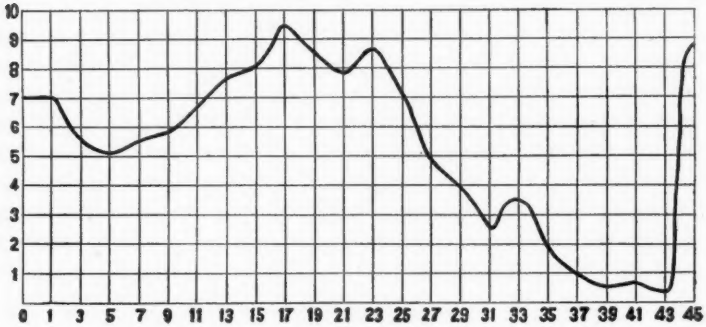

FIG. 1.—Respiration curve for seeds desiccating at 25° C.; mg. of CO_2 given off in 24 hours per gm. dry weight plotted on ordinates; time of desiccation in days plotted on abscissae; great rise in respiration after forty-third day due to placing desiccated seeds (dead at time) under favorable germinative conditions.

as buds, leaves, and embryos. They find in material taken directly from the tree increased carbon dioxide production with increased turgescence, also for decreased turgescence, and usually an increase in respiration when decrease was followed by an increase. Fig. 1 represents the trend of respiration during 43 days of desiccation. The sudden rise on the forty-fifth day shows respiratory activity of seeds after being placed in germinative conditions.

To determine the respiratory activity of germinating seeds, newly collected seeds were planted in the dark at 25° C. The respirometer used was a 500 cc. graduated cylinder. This was half filled with shredded filter paper, previously well sterilized. The

filter paper was packed very loosely in the graduated cylinder. The seeds were washed with distilled water and planted near the surface of the paper, about midway between the top and bottom of the chamber. A small amount of water was run into the respirometer. The top was stoppered and supplied with an inlet tube which extended to the bottom of the chamber and brought in the carbon dioxide free air, and with an exit tube which carried the carbon dioxide laden air to the Reiset tube. The seedlings were grown in the dark and consequently there was no food manufactured. Storage food only was used up in respiration.

The respiratory activity of the germinating seeds reaches a maximum about the eighth day at this temperature. At this time the seedling has elongated considerably, the radicle having attained a length of 7–10 cm., varying considerably with the individual. After the eighth day respiration decreases gradually. Seeds stored for several weeks at a low temperature (0° C.) and then transferred to a high temperature (25° C.) in germinative conditions show a very high initial respiratory intensity, which soon drops to normal, and then again increases. PALLADIN (**20**) found that transferring the tips of etiolated bean seedlings from a lower to a higher and also from a higher to a lower temperature increased the respiratory activity. According to APPLEMAN (**1**), tubers stored at low temperature for several weeks and then transferred to room temperature respire more intensely than tubers of the same lot not subjected to the cold storage conditions. He thinks this increased respiration might result from the increased accumulation of sugar at the lower temperatures.

Fig. 2 shows the march of respiration during the first 14 days of germination in the dark. In general this curve agrees with that found by RISCHAWI (**21**) for the respiration of the wheat seedling growing in the dark, but is quite different from that found for the bean.

Catalase activity

The apparatus used for catalase determinations was a modified form of the one used by APPLEMAN (**2**). Determinations were made upon fresh seeds, seeds desiccating at 25° C., and also seeds germinating in the dark at 25° C. Entire seeds were used in all cases.

Material was weighed, then ground in a mortar with a small amount of quartz sand and a knife point of calcium carbonate for exactly 2 minutes. This emulsion was then washed with the aid of 10 cc. of distilled water into a 200 cc. wide-mouthed bottle. The latter was then corked and plunged into a water bath kept at 25° C. The commercial form of Oakland dioxygen was used at all times. This dioxygen gives an acid reaction. To neutralize the acidity a small excess of calcium carbonate is added to the dioxygen just

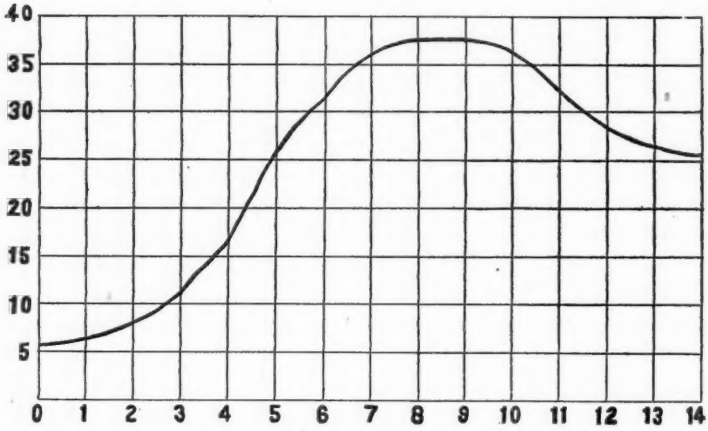

FIG. 2.—Respiratory curve for first 14 days of germination in dark at 25° C.; time of germination in days plotted on abscissae and mg. of CO_2 given off in 24 hours per gm. of dry weight plotted on ordinates.

before using. If the acidity is not corrected, the catalase activity is reduced approximately one-half. A small separatory funnel inserted in the cork of the bottle holds the dioxygen. The latter is run into the ground tissue when the dioxygen and pulp have reached the same temperature as the water bath. The material is then shaken uniformly for 10 minutes by means of a small motor. The oxygen liberated is collected over water at atmospheric pressure in a 100 cc. burette. Table II shows the catalase activity at various times during desiccation and the early stages of germination.

Catalase activity increases slightly during the first few days of desiccation, but decreases gradually thereafter. This activity seems to align itself in a general way with respiratory activity, which remained high for a considerable time. With germination the catalase activity increases enormously, appearing to be closely correlated with metabolic activity. There is not a sudden drop in the catalase activity at the time of loss of viability, as one might

TABLE II

CATALASE ACTIVITY ACCOMPANYING DESICCATION AND FIRST STAGES OF GERMINATION

CONDITION OF SEEDS OF SEEDLINGS	NO. OF CC. OF O_2 GIVEN OFF BY 1 GM. OF DRY WEIGHT IN	
	5 minutes	10 minutes
Fresh seeds collected May 25 (1918)	952	1248
Desiccated at 25° C. for 3 days.....	1035	1373
" " " " 5 "	1106	1447
" " " " 10 "	977	1341
" " " " 14 "	1075	1359
" " " " 18 "	1022	1259
" " " " 22 "	868	1098
" " " " 26 "	731	979
" " " " 34 "	688	909
" " " " 42 "	461	593
Desiccated in laboratory for 8 months..	380	500
Seedlings with radicle 1 cm. long.....	1245	1565
" " " 2 " "	1717	2055
" " " 3 " "	2106	2566
" " " 4 " "	2438	3060
" " " 5 " "	3216	4472

expect, but a gradual decrease correlated with respiratory activity and water loss. After a storage for 8 months under laboratory conditions the catalase activity was reduced more than one-half below that of the fresh seed.

Oxidase and peroxidase

Peroxidase activity is very intense in the fresh seeds. A dark blue color is obtained immediately upon addition of alcoholic solution of benzidine and a drop of dioxygen. As desiccation progresses there is a gradual decrease in peroxidase activity. In one-year-old dead seeds there is only a very pale blue color evident

about the vascular tissue when this method is used. No oxidase could be detected by the ordinary qualitative chromogenic methods in either the living or desiccated seeds.

Chemical analysis

In the following analysis seeds were collected from the same tree in order to eliminate differences due to individual variation. The collection was made in the spring of 1917. Fresh seeds were immediately placed in 95 per cent redistilled alcohol, enough being added to make the final volume of alcohol 80 per cent. One-half gram of calcium carbonate was added to guard against possible acid hydrolysis. In the final calculation the calcium carbonate was considered as being in the insoluble fraction. In general the method of extraction and analysis is that outlined by KOCH (**16**), but a few modifications were found necessary.

TABLE III

Fraction	Fresh seeds	Desiccated seeds
Percentage F_3 of total dry weight ..	79.05	65.56
" F_2 " " " " ..	15.8	30.31
" F_1 " " " " ..	5.15	4.13

The tissue was ground, and then extracted with hot 95 per cent alcohol for four hours, followed by 1-hour ether extraction. The alcohol-ether insoluble material was then heated in water for one hour on the steam bath. The water was evaporated down, alcohol again added, and returned to extraction cups for a 24-hour alcohol extraction and 1-hour ether extraction. The alcohol and ether extracts were combined, evaporated to dryness, and then extracted with anhydrous ether. This ether extract is known as F_1; the residue from the ether extract is F_2; the alcohol-ether insoluble material is F_3. F_3 was dried in the oven at 103° C. for 5 days, then cooled and weighed.

The 1917 seeds were desiccated in the laboratory. No attempt was made to maintain a constant temperature. The seeds failed to germinate after 18 days, when the water content had dropped to approximately 34 per cent. The desiccated seeds were treated in the same manner as the fresh seeds. Table III shows the

percentage variation in the various fractions accompanying desiccation.

It can readily be seen that accompanying desiccation under laboratory conditions there is a great increase in F_2. One would be led to expect quite the contrary, as condensation is quite commonly associated with desiccation in plants. Table IV shows more in detail to what this increase is due.

During the period of desiccation there has been an enormous increase in the percentage of sucrose. Accompanying this increase

TABLE IV

ANALYSIS OF FRESH AND DESICCATED SEEDS

MATERIAL	PERCENTAGE TOTAL DRY WEIGHT	
	Fresh seeds	Desiccated seeds
Free reducing sugar	0.53	0.43
Sucrose (calculated as invert sugar)	4.53	14.41
Starch	48.18	35.42
F_1 Nitrogen	0.03	0.02
F_2 Nitrogen	0.65	0.80
F_3 Nitrogen	3.36	3.28
F_1 Phosphorus	0.03	0.02
F_2 Phosphorus	0.18	0.31
F_3 Phosphorus	0.50	0.35

is a corresponding decrease in the starch content. Free reducing sugars remain approximately the same. In the desiccated seeds we also find a slight increase in phosphorus and nitrogen in F_2. The nitrogen here represents merely the Kjeldahl nitrogen.

SUGAR MAPLE (*Acer saccharum* Marsh.)

Historical

A very different type of behavior is found when the seeds of the sugar maple are considered. Germination here is initiated by a distinct period of after-ripening. Investigators generally have used the term "after-ripening" as referring to the series of chemical or physical changes occurring within the embryo or associated structures, which bring to a close the dormant period and make germination possible. The factors operating to cause delayed germination in most types of seed dormancy studied to the present

time have been treated in some detail by CROCKER (5). Seeds that have dormant periods fall naturally into two groups: (1) seeds, like certain members of the Leguminosae, have embryos capable of immediate germination, but dormancy is here induced by associated structures like the seed coats or pericarp; (2) the embryo itself may be the cause of delayed germination. The second type of dormancy may be due either to an immature embryo, as found in *Ceratozamia* (4) and *Ilex opaca* (14), the former often being shed at the time of or shortly after fertilization, while in the holly the embryo is merely a globular undifferentiated group of cells at the time of seed fall; or dormancy may appear in apparently fully matured embryos, as is the case in some members of the Rosaceae. The seeds of the sugar maple fall into the latter group, having a dormant, morphologically mature embryo.

DAVIS and ROSE (7) found that in nature *Crataegus mollis* has a dormant period of a year or more. This period of dormancy can be shortened considerably by removing the carpel and testa. It is doubtful whether any such interrelation exists between the embryo of the sugar maple and its inclosing structures.

The sugar maple sheds its fruit in the fall, after the first few hard frosts. When given the most favorable conditions for germination at time of fall the seeds fail to respond. The seeds must be kept at a low temperature, with plenty of moisture present for a considerable period of time for after-ripening to reach completion. Under natural conditions, if the seeds are kept moist during the fall and winter, after-ripening will be complete the latter part of February or early part of March.

Investigation

The object of the investigation was twofold: (1) to determine the optimum temperature and water relations for after-ripening; and (2) to determine the changes taking place within the embryo during the after-ripening period. The fruit of the sugar maple was collected the latter part of September and early part of October direct from the trees in the Chicago region and northern Indiana. Fruits were stored dry in wire baskets at various temperatures from -5 to $+30°$ C.; others were stored in desiccators over water at

5° C. and 10° C.; also, some were stored out of doors on the surface of the ground and kept covered during the fall and winter to prevent drying.

Temperature and water relations

When seeds were stored dry, in no case, regardless of storage temperature, did after-ripening reach completion; that is, no dry stored seeds would germinate when placed in Petri dishes on moist cotton at favorable germination temperatures. All dry stored seeds required a prolonged stay at low temperatures with plenty of moisture present to completely after-ripen. DAVIS and ROSE found that after-ripening in the haw proceeded best at temperatures near 5° C. The sugar maple was also found to after-ripen best at about this temperature.

In January, after three and a half months of dry storage, specimens were removed from each of the dry stored samples, and placed at 5° C. under good germinative conditions. The pericarp was removed and the seeds that had been dry stored at 5° C. were the first to complete their period of after-ripening, most of the seeds completing after-ripening during the fifth week. The seeds, however, do not after-ripen uniformly; some precede and others follow the general average time. Seeds dry stored at −5° C. take the longest time to complete their period of after-ripening, taking 4–5 weeks longer than seeds dry stored at 5° C. Seeds dry stored at 10–30° C. after-ripen more slowly than seeds stored at 5° C., and more quickly than seeds stored at −5° C. In other words, seeds dry stored at 5° C. have progressed farthest, and those stored at −5° C. have progressed least in the process of after-ripening at their respective storage temperatures. The factor limiting the complete after-ripening in the dry stored seeds at low favorable temperatures is a deficient water supply. Only in the presence of sufficient water can the various processes go progressively on to complete after-ripening.

Fruits stored on the surface of the ground were subjected to the temperature ranges of the soil surface. The seeds, however, were kept saturated, due to the extremely wet fall and winter. At time of fall seeds had a water content of 55 per cent, and during the entire fall and winter the water content remained at 55–57 per

cent. In the seeds stored out of doors and in desiccators over water there was no indication of increased water holding capacity accompanying after-ripening. Seeds stored in desiccators at low temperatures over water are completely after-ripened several weeks before seeds stored out of doors. Table V shows how after-ripening progressed in seeds stored out of doors. As after-ripening progressed, less and less time was required for the completion of this process when placed in the germinator at 10° C.

TABLE V

Put to germinate at 10° C.	Percentage of germination after number of days indicated											
	1	2	3	4	5	6	8	12	17	26	30	35
January 16, 1918	....	....	....	....	....	....	....	....	....	....	68	88
February 4	....	....	....	....	....	....	....	39	83	92	....	....
February 28	19	50	....	....	....	....	92	....	....	....	....	....
March 5	40	67	77	85	95	97	100	....	....	....	....	....

Seeds after-ripened out of doors and at 5° C. are more vigorous than seeds after-ripened at slightly higher temperatures (10° C.). Dry stored seeds at low temperatures are more vigorous when after-ripened than seeds previously dry stored at high temperatures. This question of vigor should be given more attention than it has been given up to the present time. There is something very significant in the fact that maximum vigor can be obtained by after-ripening seeds at a temperature so much below the optimum germination temperature and at a temperature which we consider retarding to metabolic activity in general. Poor germination and high seedling mortality can be replaced by good germination and vigorous seedlings when the most favorable temperature (about 5° C.) and water relations are used for after-ripening. After-ripening and germination is a continuous process, but the optimum temperature for germination is considerably above the optimum for after-ripening. Seeds completely after-ripened at 5° C. are stimulated to very rapid growth when placed at higher temperatures. On the other hand, if seeds are completely after-ripened and then allowed to desiccate at higher temperatures, seedling vigor is lowered as time progresses, and in several weeks the

embryo fails to respond when placed in favorable germinative conditions. The reason for this loss of vigor is not known. It may be due to the increased respiration, using up the plastic substances essential for the initiation of germination, or to the introduction of some new factor inhibitory to growth. After-ripened seeds placed at $-5°$ C. and kept saturated by packing in snow will retain their initial vigor for a considerable time.

Oxygen pressure

The most favorable oxygen pressure for after-ripening was not studied in detail. Seeds after-ripened in desiccators are under considerably reduced oxygen pressure. The oxygen is soon used up in respiration. Nevertheless, these seeds stored at a low constant temperature will after-ripen quicker than seeds stored out of doors with a good supply of oxygen, but subjected to fluctuating temperatures. Seeds stored in open baskets, but kept saturated at low constant temperatures, will after-ripen sooner than those stored in desiccators, and the resulting seedlings appear to be more vigorous.

Oxidase and peroxidase

ECKERSON (11) found an increase in oxidase and peroxidase activity accompanying after-ripening in the haw. In the peach CROCKER and HARRINGTON (6) found no increase in oxidase activity in the after-ripening seeds when ordinary chromogens or the Bunzel methods were used, but the pulp of the after-ripened seeds exposed to air shows a more rapid oxidation of its own chromogens. In the sugar maple there is a slight increase in peroxidase activity accompanying after-ripening, being more pronounced in the hypocotyl. No oxidase could be detected in dormant or after-ripened seeds when guaiaconic acid or benzidine was used as a chromogen.

Catalase

One of the most consistent phenomena accompanying the after-ripening of this type of embryo is the increase in catalase activity. This increase is continuous, increasing manyfold during the early stages of germination. ECKERSON (11) found that catalase activity increased in the haw with after-ripening. In

Tilia ROSE (**22**) also found a noticeable increase in catalase activity accompanying after-ripening. CROCKER and HARRINGTON conclude that "seeds that after-ripen in a germinator at low temperatures (commercial layering), in which the dormancy of the embryo is self imposed and the embryo experiences fundamental time-requiring changes for after-ripening, show a great increase in catalase activity with after-ripening (*Crataegus*, *Tilia*, *Prunus*)."

Catalase determinations were made upon the dormant and after-ripened seeds and upon the seedlings at various stages of germination. In all cases the integuments were removed and a definite number rather than a definite weight of seeds was used. The material was weighed and samples were run as described for the soft maple. The after-ripened seeds and also the seedlings used were after-ripened and germinated in the dark at 10° C. Table VI demonstrates the great increase in catalase activity accompanying after-ripening and germination in seeds of the sugar maple.

TABLE VI

STAGE	CC. OF O_2 LIBERATED BY 1 SEED OR SEEDLING IN		CC. OF O_2 LIBERATED PER GM. OF DRY WEIGHT
	5 minutes	10 minutes	10 minutes
Dormant	23.4	31.1	754
After-ripened	33.7	39.3	1117
Seedlings with 1 cm. radicle	31.0	37.0	1058
" " 2 " "	51.0	60.4	1716
" " 3 " "	87.2	98.4	2235
" " 4 " "	99.7	114.0	2230
" " 5 " "	89.2	107.0	2786
" " 6 " "	113.2	130.0	4481
" " 7 " "	125.0	142.5	4440

An increase in catalase activity is evident in both cotyledons and hypocotyl. Seeds germinated at higher temperatures also gave slightly increased catalase activity when taken at the same stage of development. Seedlings with radicles 1 cm. long were used to determine the relative catalase activity of the different parts. One-tenth gram (wet weight) of radicles, cotyledons, and integuments liberated in 10 minutes 95, 43, and 5.1 cc. of oxygen respectively. The hypocotyl, which is the most actively growing

organ at this time, gives by far the greatest catalase activity. The storage organs (cotyledons) give considerable catalase activity. The inert structures (integuments) give very low catalase activity. The difference here would be still more striking if calculated as percentage of dry weight. CROCKER and HARRINGTON find the catalase activity of wheat embryo 28–29 times that of the endosperm. The same investigators find that in grass seeds in general the physiologically inactive organs show only a small fraction of the catalase activity shown by the embryo.

Dry dormant seeds stored in the laboratory were used to determine the Q_{10} for catalase activity at temperatures ranging from 10° C. to 50° C. Seeds were ground very fine and rubbed through a 100-mesh sieve. One-tenth gram samples were used for determinations. Ten cc. of dioxygen, 10 cc. of water, and a small excess of $CaCO_3$ were added to the meal. Table VII shows the Q_{10} value for catalase activity.

TABLE VII

TEMPERATURE	Q_{10} FOR		
	1 minute	5 minutes	10 minutes
10–20° C.	1.4	1.3	1.3
20–30° C.	1.3	1.2	1.1
30–40° C.	0.1	0.9	0.8
40–50° C.	0.8	0.6	0.5

In no case does the van't Hoff law, which calls for an increase of 2–3-fold for every 10° C. rise in temperature, hold. The time consumed in heating the sample to the higher temperature introduces considerable error. The time required for complete destruction of catalase activity at any given temperature was not determined. There was still some catalase activity at temperatures slightly above 50° C. APPLEMAN (2) found the catalase activity in potato tubers to be entirely destroyed at 50° C. Between c° C. and 10° C. he finds the Q_{10} for catalase activity to be 1.5. From 10° C. to 40° C. he gets lower Q_{10} values for potato catalase than was given by the catalase of the sugar maple.

Chemical analysis

Samples were analyzed as in river maple, with slight modifications to suit the material. One-tenth gram of $CaCO_3$ was added to samples at the time of collection. Figures in the tables represent averages from several samples. Dormant seeds had made no progress in after-ripening. It is almost impossible to choose seeds for the after-ripened samples that are known to be completely after-ripened. The only criterion for completion of after-ripening is germination. The seeds in the after-ripened samples vary from completely after-ripened ones to seeds probably within a week or 10 days of complete after-ripening.

TABLE VIII

Stage	Sugar calculated as percentage to total dry weight		
	Free reducing sugar	Sucrose (as invert sugar)	Polysaccharides (as glucose)
Dormant	0.06	6.40	5.21
After-ripened	0.67	4.32	4.66
Beginning germination, radicles about 1 cm	1.81	2.36	3.43
Seedlings with 2–3 cm. radicle (with integuments)	1.13	1.80	5.91
Seedlings with 5–6 cm. radicles (integuments shed)	0.06	2.62	5.43

The protein content of the seeds is exceptionally high. The seeds contain 7.17 per cent of nitrogen or approximately 44.8 per cent protein, calculated on a dry weight basis. The embryo itself contains almost 50 per cent of protein. The nitrogen multiplied by the factor 6.25 was used to indicate the amount of protein present. The seeds contain about 17 per cent of ether extract and 11.5 per cent of total sugars. The ash percentage is relatively high, 5.87 per cent of dry weight, while 0.91 per cent of the total dry weight is phosphorus. Only a trace of free reducing sugar is present in the dormant seeds, but sucrose or sucrose-like sugars are present in considerable amounts. Table VIII shows the relative amounts of various sugars at time of dormancy, approximately complete after-ripening, and early stages of germination.

Accompanying after-ripening there is a considerable increase in free reducing sugars. Free reducing sugar reaches a maximum at the beginning of germination, and then diminishes as germination progresses. There is, no doubt, a considerable amount of sugar used up in respiration during the long after-ripening period in the germinator even at temperatures as low as 5° C. Whether the appearance of considerable amounts of free reducing sugars is merely correlated with after-ripening or is essential for the completion of after-ripening is not known. The formation of free sugars may be favored by cool uniform temperatures and high state of hydration of the embryo.

TABLE IX

Stage	Kjeldahl nitrogen as percentage of total dry weight in		
	F_1	F_2	F_3
Dormant	0.03	1.58	5.56
After-ripened	0.03	1.48	5.59
Beginning germination, radicle about 1 cm	0.03	1.63	5.29
Seedlings with 2–3 cm. radicle (with integuments)	0.03	2.37	4.73
Seedlings with 5–6 cm. radicle (integuments shed)	?	3.15	4.94

Seedlings with radicles 2–3 cm. long show an increase in polysaccharides, but a decrease in free reducing and sucrose or sucrose-like sugars. Correlated with this increase in polysaccharides is a considerable reduction in percentage of fat. The percentage of ether extract drops from about 17 per cent in the dormant and after-ripened seeds to slightly less than 14 per cent in the seedling with a radicle 2–3 cm. long. The fats in the early stages of germination are probably converted into sugar or sugar-like materials, as found in the haw by ECKERSON (11), in the sunflower by MILLER (19), and in the castor bean by DELEANO (9).

With germination there is the usual increase of the more soluble nitrogen of F_2. There is no significant change in relative nitrogen value of the dormant and after-ripened seeds. Table IX shows the relative amounts of nitrogen in the various fractions at different stages of the seeds and seedlings.

Respiration

A detailed study of respiration of the after-ripening seeds at the lower temperatures may help to interpret the metabolic activity accompanying after-ripening. Little work has been done on this phase up to the present time. Preliminary tests show very little respiration taking place in dormant air-dry seeds. When these seeds are soaked for 48 hours, however, and then transferred to the respirometer, the respiratory intensity jumps to approximately the same level as that of fully after-ripened seeds. Sufficient data have not been obtained to justify a full discussion of the correlation between after-ripening and respiration.

Hydrogen ion concentration

The gas chain method described by MICHAELIS (**18**) was used to determine the hydrogen ion concentration. Two embryos were used in each case. They were ground for 2 minutes with a small amount of pure quartz sand and 1 cc. of distilled water, and 5 cc. of distilled water was then added. This solution becomes more alkaline the longer it stands, so several readings were taken immediately and the average of these used. In both the dormant and after-ripened embryo we find a distinctly basic condition. The average of several samples shows a P_H value of 8.335 in the dormant seeds and a P_H value of 7.909 in the after-ripened seeds. Both are distinctly on the basic side of the neutral point. The hypocotyls of the dormant seeds gave a P_H value of 9.048, while that of the germinating seedlings with a 1 cm. hypocotyl gave a P_H value of 9.055. Seeds that had just started to germinate were used in the latter case, to be sure that the period of after-ripening had been completed. ECKERSON (**11**) found increased acidity in the hypocotyl of the haw with after-ripening. In working with *Tilia americana* ROSE (**22**) found increased hydrogen ion concentration with after-ripening. In the sugar maple the embryo is always basic, although the hydrogen ion may increase in concentration in the embryo when it after-ripens.

Discussion

To the present time little work has been done upon seeds that show in general the same type of behavior as found in the river

maple. Numerous observers have reported cases of seeds dying when subjected to atmospheric conditions for a short period of time. As to just what factors operate with desiccation to cause lowering of seedling vigor and early death we are still entirely ignorant. In the river maple temperature does not appear to determine the critical percentage of water loss. Death occurs at all ordinary temperatures (0–35° C.) when the percentage of water in the seeds has reached 30–34 per cent. Whether or not this will hold in general for other seeds of this type will not be known until considerably more species have been studied. In the desiccated seeds we find a noticeable increase in permeability, indicated by a large amount of sugar appearing in the substratum when placed in the germinator. The sugar makes an excellent medium for growth of bacteria and fungi, and in a few days the entire seed is completely decomposed. The fungi appear to be unable to attack potentially vigorous seeds. Whether increased permeability is the cause or the result of death is not known. Desiccation may coagulate or denature the protoplasmic proteins, increasing permeability and subsequent leaching, allowing an inroad for parasitic organisms. This type of seed stands in marked contrast to that type of seed which retains its viability best when stored in an air-dry condition. DUVEL (10) even recommends drying the majority of seeds in a vacuum or over sulphuric acid to insure good preservation. In fact, many seeds can be dried to constant weight without lowering viability or seedling vigor. KIDD (15) states: "In the case of certain rapidly deteriorating seeds (*Hevea brasiliensis*) the carbon dioxide naturally produced by respiration of the seeds in a closed flask rose to 40 per cent, and the pressure of this was found to be accompanied by a marked prolongation of vitality in the seeds. This prolonged vitality was far in excess of that reached with the present commercial method of packing these short-lived seeds for export." Where there is a rapid oxidation of food material due to high respiration, there is no doubt that narcotizing the embryo would result in greatly reduced metabolic activity. Whether or not high embryo vigor can be maintained in the river maple by narcotizing still remains to be determined. Storage at 0° C. over water, however, provides an excellent condition for the seeds of river maple.

Recent studies have thrown considerable light upon the behavior of seeds that require a definite time under certain favorable conditions to after-ripen a morphologically mature embryo. The major portion of the work up to the present time has been done upon various members of the Roseceae. No doubt seeds of this general behavior exist in many more of our plant families, especially among the uncultivated forms. Not until more work has been done upon a wider range of plants will it be known just how widespread this phenomenon is. The few species studied thus far by various investigators show remarkable similarity of behavior in several features accompanying after-ripening. There are five more or less specific changes, according to CROCKER and HARRINGTON (**6**), which are quite conspicuous in the constant way which they seem to accompany after-ripening in seeds of this type: (1) rise in vigor of seedling, (2) increase in amount of water absorbed, (3) increase in total acidity, (4) increase in catalase, and (5) oxidase activity.

When after-ripening is accomplished under the most favorable conditions of oxygen pressure, water relations, and temperature, seedling vigor is in all cases at its maximum. In the sugar maple, at least, seedling vigor can be judged only during the first stages of germination after the completion of the period of after-ripening. After-ripening, however, may complete itself under conditions not favorable for the greatest expression of seedling vigor.

ROSE found slight increase in acidity accompanying after-ripening in the seeds of *Tilia*. This was correlated with greater water holding capacity. In the haw (**11**) delayed germination of the embryo has been found to be due to a dormant hypocotyl. In the dormant seed this organ is slightly alkaline or neutral, but with after-ripening the hypocotyl becomes distinctly acid. Accompanying this increased acidity there is increased water holding capacity of the hypocotyl, along with increased activity of the enzymes. Here the hydrophilous colloids have a greater water holding capacity in a slightly acid medium. When the entire seed of the haw is considered, however, we find a slightly higher water holding capacity in the dormant than in the after-ripened seed. In the sugar maple the water holding power of the hypocotyl only was not determined. Considering the hydrogen ion concentration

found in the hypocotyl of the dormant and after-ripened seeds, one would hardly expect to find a change in the water holding capacity of the hydrophilous colloids. Determinations on the water content of entire seeds stored in favorable after-ripening conditions show that there is no change in the water holding capacity of the seeds as a whole.

One of the most consistent phenomena accompanying after-ripening in this type of embryo is the great increase of catalase activity. This appears to be an accompanying feature of more than ordinary importance. A large number of investigators in various branches of animal and plant physiology attempt to correlate catalase activity with metabolic activity in general. BURGE (3), by increasing the work of certain fowl muscles and consequently the respiratory and metabolic activity, has made the catalase activity increase enormously. In the castor bean DELEANO (9) found a rapid increase in catalase activity at the beginning of germination. A great increase in catalase activity accompanied germination in the sugar and river maples. In the fully imbibed seed of Johnson grass, CROCKER and HARRINGTON (6) found catalase activity paralleling respiration. This did not hold for seeds of the amaranth, however. In the potato, APPLEMAN (1) found respiratory and catalase activity closely accompanying each other. ECKERSON (11) found an increase in the catalase activity with after-ripening in the haw. An increase in catalase activity with after-ripening has also been reported for *Tilia americana* (22). In the sugar maple there was a 66 per cent catalase activity increase in the after-ripened seeds over that of the dormant seeds. Just how closely catalase activity and respiration parallel each other during the course of after-ripening has not yet been determined. From evidence at hand showing the almost universal correlation of these two phenomena we might reasonably expect to find respiration increase noticeably during the process of after-ripening. Respiratory activity should be determined continually throughout the entire period of after-ripening at the temperature and water relations most favorable for after-ripening. Preliminary respiratory determinations reported in this paper are not conclusive. The seeds were transferred from 5° C. to the 20° C. oven. This change

in temperature no doubt introduces changes which may possibly mask the real condition at the lower temperature.

Accompanying after-ripening in the sugar maple is an increase in the amount of free reducing sugars. Just how generally this occurs in this type of embryo is still unknown. Whether increase in amount of free reducing sugar is essential for the completion of after-ripening is problematical. Dormancy is probably due to a temporary suppression in the development of one factor or a group of factors essential for the normal functioning of the embryo in germination. It is impossible to select any one factor as the cause of dormancy in the embryo of the sugar maple at the present time. Whether any certain observed change in the embryo accompanying after-ripening is responsible for bringing dormancy to a close, or whether this change results merely from the conditions to which the embryo has been subjected, remains a question.

Summary

River maple

1. Seeds lose their viability when the water content is reduced to 30–34 per cent.

2. Temperature seems to play no part in determining the critical point of water loss. Higher temperatures only hasten the rate at which the point of desiccation is attained.

3. Respiratory activity in the desiccating seeds at 25° C. first decreases slightly, then rises to a maximum, then gradually falls to zero as desiccation progresses.

4. After a slight initial increase, catalase activity gradually decreases in the desiccating seeds. Catalase activity increases enormously during the early stages of germination.

5. Seeds of a river maple may be kept in a vigorous viable condition for a considerable period of time at low temperatures (0° C.) stored over water.

6. There is a gradual decrease in peroxidase activity accompanying desiccation.

Sugar maple

1. Seeds after-ripen best at temperatures near 5° C., with a good supply of oxygen and moisture.

2. With after-ripening the seeds show a considerable increase in free reducing sugars.

3. Catalase activity increases greatly with after-ripening and germination; there is also a slight increase in peroxidase activity.

4. Both the dormant and after-ripened seeds have a reaction that is distinctly alkaline; this holds for the hypocotyl as well as for the entire embryo.

5. Fully after-ripened seeds will remain in this condition for a long time if kept moist at $-5°$ C.

Acknowledgments are due Dr. CROCKER and Dr. ECKERSON, through whose efforts and encouragement this piece of work was made possible. Many thanks are due also to Dr. T. G. PHILLIPS, who very kindly made the hydrogen ion determinations.

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POLYEMBRYONY AMONG ABIETINEAE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 261

JOHN T. BUCHHOLZ

(WITH FIFTEEN FIGURES)

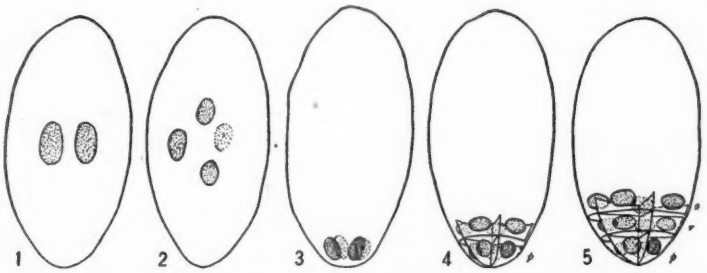
Polyembryony among conifers is of two kinds: cleavage polyembryony, in which a single fertilized egg gives rise to many embryos; and the simple polyembryony, which is due to plurality of archegonia. This latter form is encountered wherever there are several eggs that may be fertilized, and therefore is found among all gymnosperms. The fact that polyembryony was found in both the pines and the cycads, and was due to plurality of "corpuscula" or "areolae" (archegonia) in both instances, was one of the arguments presented by BROWN (**1, 2**) as early as 1826 as showing a fundamental relationship between these two groups.

A form like *Pinus*, which has cleavage polyembryony, usually has several eggs fertilized also, and therefore combines both forms of polyembryony. Since each zygote in *Pinus* usually gives rise to a system of 8 embryos, there may be as many embryos as 8 times the number of fertilized eggs. If all 6 of the archegonia of some species were fertilized, 48 embryos might be produced, but 4 is the maximum number of embryo systems that have actually been found, and even then many of the embryos disappear very early, some of the rosette embryos being aborted without division of the embryo initial cell.

In discussing polyembryony, it is necessary to consider briefly the pine proembryo stages, shown in the accompanying figures. The writer's interpretation of the facts brought out by various investigators, together with his own studies, would describe the initial steps in the development of the pine embryo as follows.

The zygote begins development with free nuclear divisions (figs. 1–3). When 4 free nuclei have been formed they descend to the bottom of the egg, and there undergo another free nuclear division, after which the primary embryo initial group of cells (*p*) is

cut off by complete walls from the rest of the cytoplasm of the egg. Each cell of this tier constitutes an initial cell to one of the 4 primary embryos. The tier above it is not completely walled, and therefore undergoes another free nuclear division, organizing the second tier of completely walled cells (*r*), the rosette tier, a group of initial cells of the rosette embryos. The open tier of free nuclei (*o*) which remain above this undergo no further division and soon disintegrate. When these 3 tiers of 8 walled cells and 4 free nuclei have formed, as in fig. 5, the organization stage of the proembryo is concluded, for each cell is now ready to produce its own distinct embryo, although the 4 cells of the primary embryo initial tier (*p*) continue their further development in unison.

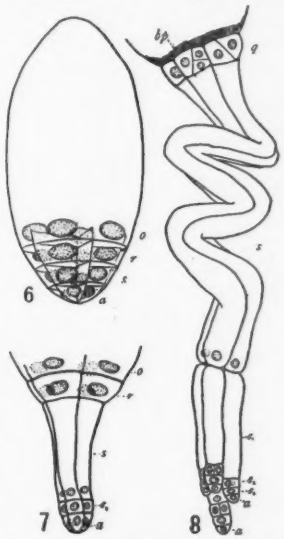


FIGS. 1–5.—Steps in development of proembryo in *Pinus*, diagrammatic reconstructions from serial sections and published figures: *p*, tier of primary embryo initial cells; *r*, tier of rosette cells, initial cells of rosette embryos; *o*, upper open tier of cells; normally tiers *r* and *o* come from division (free nuclear) of upper tier of fig. 4.

From each of these 8 completely walled embryo initials (fig. 5) an embryo develops by means of an apical cell, this cell functioning first as a hemispherical apical cell of one cutting face, and later as a semi-pyramidal cell of 3 cutting faces, in a manner described in greater detail elsewhere (3). It may be added that this apical cell persists until an embryo mass of about 500 cells has been formed, after which it is replaced by the meristematic group of cells found in the older conifer stem tip. This apical cell is a primitive feature in which conifers recapitulate their fern phylogeny.

THE EARLY EMBRYO OF PINUS.—The cells (*p*) of the embryo initial undergo simultaneous division, in which their first apical

cell segments (*s*), the primary suspensor cells, are cut off. This group constitutes what has generally been recognized as the suspensor tier of the 16-celled stage (cf. figs. 5, 6). Next the suspensor cells (*s*) elongate and thrust the embryonal tier of apical cells into the pocket which the digestive enzymes of the eggs and embryos have corroded within the gametophyte, the 4 embryo units separating and their apical cells (*a*) continuing to give rise to segments (e_1, e_2, etc.), which elongate and add to the suspensor.



FIGS. 6–8.—Stages in development of early embryo in *Pinus*: *a*, apical cells; *s*, primary suspensor cells; *r*, rosette cells, which give rise to *q*, rosette embryos (latter usually develop later than in stage of embryo shown); e_1, e_2, etc., embryonal tube initial cells and embryonal tubes, which elongate and add to suspensor; diagrammatic reconstructions.

Soon the rosette group of initials divides and the development of the rosette embryos is begun (*q*, fig. 8). It will be seen, therefore, that not only do these 8 embryos per zygote all result from free nuclear cleavages, but the several embryos develop independently from the time the first walls are organized. The primary embryos develop without interruption from their initials, while the rosette embryos are delayed, developing somewhat later, on an average, than is indicated in fig. 8. In the hundreds of instances that have been examined in my investigations of various pines, none were found where the 4 primary embryos were combined to produce a single embryo, nor were any cases found where one of the primary embryos was further split up to give rise to 2 or more embryos.

In the competition which ensues, the rosette embryos play a very subordinate rôle, owing to their unfavorable position and delayed development. Among the 4 primary embryos, the competitive process elects one embryo from the complex, nearly always the embryo which develops the longest suspensor, pushing it ahead of

its competitors. Embryonic vigor in producing a long suspensor is the outstanding factor which decides upon the successful embryo. The mass of embryonal tubes which elongate from the base of the embryo, as this and the suspensor become more massive, doubtless assist the successful embryo in checking the others. Usually it is the embryo foremost in position which is successful in developing to maturity, but sometimes the second one in position becomes massive more rapidly and assumes the leading rôle, by choking out the smaller terminal one. Not only must an embryo have a rapidly developing suspensor, but it must also become many-celled and massive more quickly than any of the competing embryos.

Vigorous suspensors have been the basis of selection among the embryos of gymnosperms for so long a period that this organ has become a large and extensively developed structure, many times larger than would be necessary without this embryonic competition. This is true whether the competing embryos come from the same egg, as in cleavage polyembryony, or the selection occurs between neighboring zygotes, as among cycads. The remarkably long suspensor found in nearly all gymnosperms has always been a noteworthy feature of this group.

Investigation

OTHER PINE SPECIES.—The result of a further investigation of the embryo development in various species of pines confirmed the account as announced for *Pinus* (3). The additional work done on *Pinus Strobus*, *P. ponderosa*, *P. edule*, and *P. resinosa*, as well as a further examination of *P. Laricio*, *P. Banksiana*, and *P. sylvestris*, makes it practically certain that cleavage polyembryony, the apical cell development, and the rosette embryos are found quite constantly among all members of this genus.

It might be noted that *Pinus sylvestris* seems to have a marked tendency to produce shorter suspensor cells and embryonal tubes than *P. Banksiana*, which was taken as the type for the previous investigation. In *P. Laricio* the 4 primary embryo units frequently do not split apart until the primary suspensor cells have stretched to about half their final length and the first embryonal tubes are beginning to elongate. Indeed, when some of these earlier stages

were examined, the writer's prediction was that in this species, at least occasionally, the usual separation into 4 primary embryos did not occur, but hundreds of embryos dissected out in slightly later stages (several days older) of material from the same source failed to reveal even one case without the usual cleavage polyembryony.

The rosette embryos of *Pinus Laricio* are very clear. In many cases they have suspensors which elongate distinctly, and were it not for the fact that the dissections clearly show their relation to the basal plate (*bp*), these rosette embryos would in some instances very easily be confused with the primary embryos. On the whole, the embryos of *P. Laricio* furnish probably the most satisfactory type for use in laboratory instruction, both on account of their clearness in displaying the rosette embryos, and their large size, which makes them easier to dissect.

ABIETINEAE.—The other genera of Abietineae that were dissected and examined are *Cedrus libani*, *Tsuga canadensis*, *Abies balsamea*, *Picea mariana*, *Picea excelsa*, *Larix europea*, and *Pseudotsuga taxifolia*, the species investigated representing 7 out of the 9 genera of the Abietineae.

METHOD AND MATERIAL.—The technique was that of dissection described in detail in the writer's work on *Pinus*. No modifications of these methods were found necessary, but perhaps it should be repeated that the living material is indispensable for some species. A study of preserved material is possible, but it is not so satisfactory. The embryos may be killed and preserved indefinitely, however, after they have been removed by the methods described. The proembryo stages must be studied by the well known methods for making serial sections. The writer is indebted to the following for the material used during the summer of 1917: W. G. WATERMAN for material of *Abies* and *Tsuga* from Frankfort, Michigan; S. D. MAGERS for collections of *Abies balsamea* and *Picea mariana* from Marquette, Michigan; D. Hill Nursery Company, of Dundee, Illinois, for material of *Pseudotsuga*, *Larix*, and *Tsuga canadensis*, collected on their grounds. Very satisfactory material of *Pseudotsuga taxifolia* was supplied by the Friday Harbor Marine Station of Puget Sound. During June and July C. T. HILMERS supplied

weekly collections of the material growing on the University Farm near Lincoln, Nebraska, as follows: *Picea excelsa*, *Pseudotsuga taxifolia*, *Pinus ponderosa*, *P. sylvestris*, *P. Laricio*, and *P. Strobus*. In addition to this, the writer made many trips to various places in the vicinity of Chicago to secure material of some of these same species. During the summer of 1918, W. W. ROBBINS supplied a collection of *Pseudotsuga taxifolia* from near Fort Collins, Colorado, and arranged for a collection of *Pinus edule* from Cortez, Colorado; and E. J. KRAUS made several collections of the cones of *Cedrus libani* from the grounds of the Oregon Agricultural College, Corvallis, which reached the writer in excellent condition.

Cedrus has almost the same early embryogeny as *Pinus*. The primary embryos, however, do not separate until some time after the suspensor cells and first embryonal tubes have both elongated, and therefore cling together very much longer than in any species of *Pinus* that was investigated. In all the slightly older stages the embryo units had separated, indicating that cleavage polyembryony is likewise a constant feature in *Cedrus*. An apical cell stage seems to exist in this genus, and rosette embryos usually occur, somewhat less developed than in the average pine. The older suspensor cells collapse soon after separation of the primary embryo units.

Tsuga canadensis also resembles *Pinus* very much in its embryogeny. In this species the embryo units separate into the 4 primary embryos, yet they cling together longer than in any pine, apparently about as long as in *Cedrus*. Cleavage polyembryony occurs regularly. This conclusion is based upon the careful dissection and examination of the embryos of about 40 ovules of a more advanced stage, among which no exceptions were found.

Save for their difference in size, *Tsuga*, *Cedrus*, and *Pinus* appear very similar in the first stages of suspensor formation. In *Tsuga*, however, the rosette cells are very ephemeral; they were not found to divide before the collapse and disintegration of their contents, apparently giving no rosette embryos. The suspensor cells also collapse very soon in *Tsuga*, leaving only a shred of tissue which connects the shriveled rosette to the embryo system below. As in *Pinus*, the early embryos develop by means of an apical cell.

There are from two to four archegonia present in *Tsuga*, and in the material studied one or two embryo systems was the usual number found. The cones were very poorly pollinated, and doubtless the normal maximum number did not occur. Polyembryony, although extensive, is much less pronounced than in *Pinus*, for in addition to the small number of archegonia, there are no functioning rosette embryos.

In *Abies* the normal product of a fertilized egg is a single embryo. The group of rosette cells is present, and in a few rare instances a divided rosette cell and a more advanced rosette embryo were found. This, as well as the fact that cleavage polyembryony was also observed in a few cases, shows that this genus stands next to *Cedrus* and *Tsuga* in its similarity to *Pinus*.

The apical cell stage is doubtless eliminated from the beginning, for when under normal conditions all of the lower tier of cells combine to produce a single embryo, the terminal cells together are responsible for producing the tissue. It appears also from an examination of some of the early embryos that these 4 terminal cells of the apical group do not always contribute equally to the cell mass, for one of these 4 terminal cells may frequently be found decidedly more prolific than the others. Normal apical cell growth, however, is not possible unless cleavage polyembryony occurs, as it rarely does.

The suspensor cells and upper embryonal tubes of the secondary suspensor collapse very soon after elongation. The basal plate (*bp*), a deposit formed within the egg over the rosette cells, is very thick and frequently obstructs a clear view of the rosette cells, which also collapse early, unless a rosette embryo happens to develop.

The material of *Picea* was somewhat limited. The cones that could be secured of *P. mariana* were younger than the fertilization stage, and a later collection was too old for a satisfactory study of the early embryo. A number of twigs bearing cones from the first collection were kept in a tin box in the laboratory for more than a week, and at the end of this time they were found to contain embryos in the desirable stages. The *P. excelsa* cones were very poorly pollinated, and only a few good embryos were secured from

this species. A study of this material makes it clear that cleavage polyembryony does not occur, but each archegonium produces only a single embryo. The group of rosette cells is present, but no divisions were found within these cells producing rosette embryos, as they do occasionally in *Abies*. *Picea*, therefore, is a step farther removed from *Pinus* in having eliminated all traces of cleavage polyembryony and rosette embryos, except the tier of rosette cells.

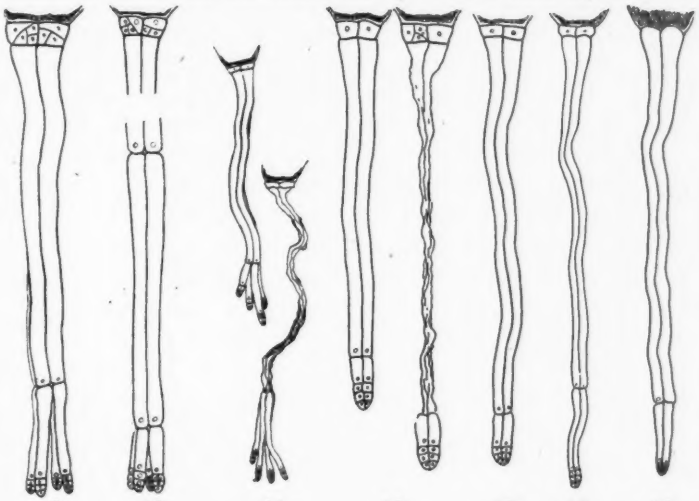
Although the available material of *Larix* was also somewhat limited, several outstanding features may be described with certainty. Like *Picea* and *Abies*, only one embryo is produced per archegonium. Except for the different appearance in size and proportion, the embryo of *Larix* is very similar to that of *Picea*. The 4 collateral primary suspensor cells become very long and slender, without the abrupt twists or turns found in the pine suspensor, and the secondary additions of the suspensor have similar characteristics. The older divisions of the suspensor collapse as the newer embryonal tubes elongate from the base of the embryo. A group of rosette cells is present, but these collapse without forming embryos, and the basal plates are again large, obstructing a good view of the former in many cases.

Pseudotsuga furnishes a rather interesting variation from the embryos already described. This form is like *Picea* and *Larix* in producing only one embryo from each egg. It has no rosette cell, but the uppermost tier of walled cells elongates to form the suspensor, a condition shown in less than 5 per cent of the pine embryos (*Pinus Banksiana*). This occurs as a regular feature in *Thuja* (12) and many other conifers. As the suspensor elongates, the contents of the archegonia shrink and harden, and persist as flattened, deeply stained structures attached to the upper ends of the transparent suspensors. A very thick layer of protoplasm or other substance, in the position which corresponds to the basal plate, stains more deeply than the remaining regions of the withered archegonia. Although cleavage polyembryony does not occur, a larger number of embryos is produced than in *Abies*, *Larix*, or *Picea*. This is due to the existence of a larger number of archegonia, which range from 5 to 8. The suspensor cells do not collapse early, as in *Larix* and *Abies*, and although the embryos were never found splitting into

separate units, the suspensor cells back of the embryo become easily separated from each other.

Discussion

It will be seen that among the 7 genera of the Abietineae examined, the last three do not possess cleavage polyembryony even as an occasional feature, while in *Abies* it occurs only in rare instances. Likewise the rosette embryos occur normally in *Pinus*



FIGS. 9–15.—Embryos of 7 genera of Abietineae, showing intergrading series with cleavage polyembryony on the one hand (figs. 9–11) and its absence on the other (figs. 12-15); rosette embryos in *Pinus*, *Cedrus*, and occasionally *Abies;* diagrams not drawn to scale.

and *Cedrus*, and only rarely in *Abies*, while none of the other forms shows them even occasionally. *Cedrus* and *Tsuga* are most like *Pinus* in possessing cleavage polyembryony as a constant feature, but in the latter the rosette cells do not produce rosette embryos. Rosette cells, even though they produce no embryos, as in *Tsuga*, *Larix*, and *Picea*, are clearly homologous with these embryo initials in *Pinus* and *Cedrus*, and represent vestigial structures wherever they are present. Figs. 9–15 illustrate these differences. We have

here a very interesting intergrading series, with *Pinus* at one end and *Pseudotsuga* at the other. There seem to be but two alternatives; either the *Picea* or *Pseudotsuga* type of embryo has given rise to the *Pinus* type with cleavage polyembryony, or the *Picea* embryo is composite in its origin, being made up of the fused or combined elements that produce the many cleavage embryos in *Pinus*.

The writer believes that the pine embryo with its cleavage polyembryony is the primitive type, and the following are among the reasons for this conclusion. The pine embryo combines with cleavage polyembryony the apical cell, a primitive character, which clearly recapitulates its semi-pyramidal predecessor at the stem tip of the fern. To assume that cleavage polyembryony is a derived feature would take away all phylogenetic significance from this structure, for the *Picea* and *Pseudotsuga* type of embryo have no apical cell. The apical cell could hardly be considered an accidental result of the splitting of a *Picea*-like embryo. This conception might be entertained if the terminal cell began to display apical cell characteristics only after separation of the embryos, but a true apical cell has been shown to exist from the embryo initial stage, from the time the first walls appear in the proembryo.

The apical cell is present in the adult ferns and in the first stages of the pine embryo; it is absent in all adult gymnosperms and likewise in angiosperms. This structure has been eliminated in passing from the lower to the higher vascular plants, and in *Picea*, *Larix*, and *Pseudotsuga* the apical cell is entirely eliminated from the beginning of the life history. The embryo development in this group shows how the apical cell was lost in the evolution of the Abietineae.

Another reason why the *Pinus* embryo must be considered the more primitive type arises from the study of the rosette embryos. In the *Picea* embryo are found the vestigial rosette cells, which never divide, but are clearly homologous with the rosette embryo initials in the pine. Even in the pine these rosette embryos are vestigial, but since these rudimentary structures are well developed in the latter, one would infer that the *Pinus* type represents the more primitive condition.

Another point in favor of the view that cleavage polyembryony is a primitive feature is the fact that *Pinus* is known to be very old historically. This genus has come to be regarded by paleobotanists as one of the very oldest conifers (**6**). On the other hand, JEFFREY (**9, 10**) has reached this same conclusion on the basis of anatomy.

An additional argument that cleavage polyembryony is primitive comes from a consideration of the relation that the pine embryo holds to the known steps in the embryo development of other conifers. There are several lines of evolution which have arisen from a primitive type of embryo like *Pinus*. One of these is the abietineous evolution shown in this investigation, the series beginning with *Pinus* and culminating in *Pseudotsuga*. Another evolutionary series begins with *Pinus*, involves some of the Cupressineae and Taxodineae, and culminates in Gnetales, a line in which cleavage polyembryony has been retained. *Ephedra* has a modified form of cleavage polyembryony, which associates it with Coniferales on the basis of its embryogeny. Other evolutionary lines may have been derived from the *Pinus* type of embryo, as described elsewhere (**3**). This is therefore another strong argument that the pine type of embryo is very primitive.

STRASBURGER (**18**) has reported that *Picea* develops only one embryo per archegonium, and his results are thus verified by this study, but he did not attach any significance to the question of whether or not a separation of the embryos occurs. Other investigators in dealing with the embryos of the Abietineae have likewise failed to make this point clear, and the embryogenies of some genera, such as *Cedrus*, *Tsuga*, *Abies*, and *Larix*, have been partially investigated in proembryo stages only.

The proembryo of *Pinus* has been most extensively studied, described, and figured by CHAMBERLAIN (**4**), COULTER and CHAMBERLAIN (**5**), Miss FERGUSON (**7**), and Miss KILDAHL (**11**), each investigator adding a few additional stages and details. The facts brought out by these investigators are in harmony with the interpretation given to the proembryo in this paper.

The embryogeny of conifers has not usually been undertaken by morphologists as a distinct problem, but the stages described and

figured were often rather incomplete, being only the by-product of another investigation. In several instances the proembryo of other Abietineae has been described as being the same as *Pinus*, but it is doubtful if all of the investigators verified every step of the embryogeny included in their account. Four tiers of 4 cells (fig. 6) may be produced by several methods of division.

LAWSON (13) describes 4 tiers of 4 cells each for *Pseudotsuga*, but since this species has no rosette group, the exact order of division and the stages corresponding to figs. 4–7 in *Pinus* may not be the same. The writer has not had opportunity to examine the proembryo or the earliest stages of the embryo in this species, but it may be inferred that one of two things happens in the *Pseudotsuga* embryo. Either the lowest tier, shown for *Pinus* in fig. 4*p*, continues to divide to give rise to the additional two tiers of cells, or, more probably, the exact order of division shown in *Pinus* is carried out, and it is the rosette tier which elongates. *Pinus Banksiana* (3) was found with elongated rosette cells in nearly 5 per cent of the cases studied. It is very important, therefore, to know whether the divisions that occur in the proembryo of any species are homologous with those of *Pinus*.

MIYAKE (14), in his study of *Picea*, includes the stages of the proembryo, and fortunately he figured a stage between fig. 4 and fig. 5, also between fig. 5 and fig. 6, which proves that the rosette tier found in this form is identical in origin with that of *Pinus*, and the rosettes of these two species are therefore distinctly homologous.

Tsuga and *Abies* probably have proembryos identical with *Pinus*, in view of the results shown for *Picea*. Only a few stages of the proembryo in *Tsuga canadensis* are definitely known. These were figured by MURRILL (17) as essentially the same as *Pinus*, but not illustrated in stages older than fig. 3. *Abies balsamea* was shown by MIYAKE (15) to be practically the same as *Pinus* for the stages up to and including fig. 4. In view of the similarity of *Pinus* and *Cedrus* in their early embryogeny, there can be little doubt that the proembryo of the latter develops in very much the same manner.

Only two genera of the Abietineae have not been investigated in some early stage by the writer. These are *Keteleeria* and *Pseudo-*

larix. The later embryo and other anatomical features of *Keteleeria* are described by HUTCHINSON (**8**), but the early embryo still remains to be studied. *Pseudolarix* was described by MIYAKE and YASUI (**16**), whose work shows stages in the embryo similar to figs. 2, 4, and 6, with a figure showing the suspensor cells beginning to elongate. This species has rosette cells and appears more slender, but is otherwise like the average of the Abietineae in the same stage of development before the embryo units separate (if they do). This embryo is not like *Pseudotsuga*, therefore, but probably belongs somewhere in the series (figs. 9–15) between *Tsuga* and *Picea*, the exact position depending upon whether or not cleavage polyembryony occurs, and whether the rosette cells give rise to rosette embryos.

Some taxonomists include *Pseudotsuga* in the same genus with *Tsuga*. The results of this investigation show that, on the basis of the embryogeny at least, there is a fundamental difference between these two forms, which would entitle *Pseudotsuga* to be recognized as a separate genus. The contrasting differences may be summarized as follows. *Tsuga* has cleavage polyembryony and apical cell growth in its life history, while *Pseudotsuga* has none of these features; and while the rosette cells do not produce embryos in *Tsuga*, they are either entirely absent in *Pseudotsuga* or they elongate to form the suspensor and are not recognizable. The latter genus has also 5–8 archegonia, while *Tsuga* usually has a smaller number (2–4).

It should be noted that the difference between the embryo of *Pseudotsuga* and *Tsuga* is greater than that between *Abies*, *Larix*, and *Picea*, and much greater than that between *Pinus* and *Cedrus*. *Cedrus*, on the other hand, shows little in its early embryogeny which would entitle it to a place as a separate genus, but the difference between *Pinus* and *Cedrus* is nearly as great as that between *Larix* and *Picea*.

Summary

1. Although all species of *Pinus* have shown a complete separation of the 4 primary embryos, this feature of cleavage polyembryony is not characteristic of all Abietineae.

2. The cleavages which separate the 8 embryos from each other are the free nuclear divisions of the proembryo. In forms without cleavage polyembryony (*Picea*, and as far as we know concerning other forms), cell divisions homologous with those in *Pinus* occur in the proembryo.

3. The embryos of the Abietineae may be arranged in an intergrading series, with *Pinus* at one end and *Pseudotsuga* at the other, on the basis of the occurrence of cleavage polyembryony, rosette embryos, and the apical cell. The rosette embryos and their vestiges, the rosette cells, are gradually eliminated as we pass from *Pinus* to *Pseudotsuga*.

4. Cleavage polyembryony, rosette embryos, and the apical cell mark a primitive type of embryo development.

5. The embryo development of this group shows how the apical cell was lost in the evolution of the Abietineae.

6. On the basis of embryogeny *Pseudotsuga* is unique and is entitled to rank as a separate genus.

This study was begun at the Hull Botanical Laboratories in the summer of 1917 and is the result of a preliminary study of the embryo material of these conifers. More detailed descriptions of the embryos with illustrations will appear later. The writer takes pleasure in acknowledging his indebtedness to Dr. C. J. CHAMBERLAIN for valuable council in getting this investigation under way.

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CHEMICAL AND PHYSICAL CHANGES DURING GEOTROPIC RESPONSE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 262

THOMAS G. PHILLIPS

Introduction

The work reported in this paper was undertaken with the object of making as complete a study as possible of all the chemical and physical processes that might be involved in geotropic response. It was hoped in this way not only to add something to the knowledge of the mechanics of geotropic bending, but also to find some quantitative differences which are associated with the differing rates of growth of the two flanks of the responding organ. It became necessary to drop the work before it was complete. Such results as were obtained are reported in the hope that they may prove of some value to others interested in the problem.

Several studies of one or more of the factors which might be involved have been made. KRAUS (**8**) found that the water content of the convex flank of organs stimulated geotropically is greater even before bending begins. He also made determinations of reducing sugars and titration acidity on the juice expressed from the organs. He concluded that when a stem capable of negative geotropic response is laid horizontally, increased sugar formation begins at once, and the amount of free acid decreases. This occurs especially on the lower side. There is a movement of water from the upper to the lower side. Thus the concentration of sugar in the juice of the lower side becomes less than in that of the upper.

Miss SCHLEY (**9**), working with shoots of etiolated *Vicia Faba* seedlings, found rather complex changes in the titration acidity after exposure to gravity. First the concave side was more acid, then the convex, then they became about equal while bending was in progress. After the tip had passed the vertical, the concave side became the more acid, but this difference gradually disappeared. She found the water content somewhat greater on the convex side,

but the samples were taken after bending was practically complete. The percentage of sugar in the convex flank was considerably lower than in the concave, after an exposure of 45 minutes.

In various roots exposed to gravity CZAPEK (3) found an accumulation of intermediate products of oxidation of certain amino acids, due to the presence of an antienzyme which inhibits the normal oxidation of these substances. He found no differences between the upper and lower flanks in this respect. GROTTIAN (7) and GRAFE and LINSBAUER (5) were unable to confirm CZAPEK'S results. The latter workers (6) found that geotropic response causes no differences in catalase activity.

SMALL (10) found increased permeability in the cortical cells of both sides of root tips of *Vicia Faba* when exposed to gravity. The permeability of the lower sides showed a greater increase than that of the upper side.

Changes in the viscosity of the protoplasm during geotropic stimulation were studied by WEBER (11), who found that the viscosity is lessened. ZOLLIHOFER (12) was unable to confirm this result, and states that the method used is subject to large experimental errors.

Experimental work

The first material used in this work was nodes of corn that had completed their growth. The node was cut out, together with about half the internodes above and below, and the sheath removed. The node was then planted horizontally in a bank of moist sand in a box from which light was excluded. This material is especially good because no growth occurs aside from that due to the action of gravity, and because the region which bends in most cases is very clearly defined. After exposure to gravity this region was cut out and divided into upper and lower flanks. There are at least two objections to the use of corn nodes. First, suitable material can be obtained only during a comparatively short time each year. Second, whether a given node will respond to gravity is very uncertain. Some nodes that apparently were healthy and in good condition did not respond at all, and others which showed no evident differences responded readily. This makes practically impossible a study of the period before visible bending begins.

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Etiolated *Vicia Faba* seedlings were used for the later work. For the moisture and titration acidity determinations the plants were grown in moist sphagnum in pans. When the shoots had reached a suitable length (6–8 cm.) they were exposed to gravity by setting the pans on edge. In collecting the material, the leaf was removed and the stem divided as accurately as possible into upper and lower flanks. The terminal 3–4 cm. were used. For the other work the plants were grown in moist sawdust in a dark cool room.

TABLE I

MOISTURE AND ACIDITY IN CORN NODES EXPOSED TO GRAVITY

TIME OF EXPOSURE	MOISTURE			ACIDITY IN CC. 0.05 *N* NaOH PER GM. FRESH WEIGHT		
	Upper flank (percentage)	Lower flank (percentage)	Difference (percentage)	Upper flank	Lower flank	Difference
Hours						
3.....	86.68	87.10	+0.42	0.49	0.48	−0.01
3.....	87.00	86.83	−0.17	0.47	0.47	.....
6.....	86.85	86.98	+0.13	0.47	0.53	+0.06
6.....	87.09	87.18	+0.09	0.51	0.59	+0.08
9.....	84.97	84.43	−0.54	0.46	0.48	+0.02
9.....	84.04	85.10	+1.06	0.51	0.54	+0.03
12.....	83.80	83.19	−0.61	0.49	0.50	+0.01
12.....	83.10	82.72	−0.38	0.57	0.59	+0.02
15.....	85.50	84.61	−0.89	0.47	0.46	−0.01
15.....	84.24	84.26	+0.02	0.47	0.54	+0.07
18.....	83.50	83.71	+0.21	0.38	0.48	+0.10
18.....	82.39	82.79	+0.40	0.51	0.55	+0.04
21.....	82.35	83.40	+1.05	0.61	0.65	+0.04
21.....	82.31	82.71	+0.40	0.67	0.70	+0.03
24.....	83.73	82.99	−0.74	0.55	0.57	+0.02
24.....	82.97	82.90	−0.07	0.58	0.65	+0.07
27.....	81.39	82.19	+0.80	0.64	0.57	−0.07
27.....	81.44	82.44	+1.00	0.65	0.76	+0.11

When they had reached a suitable length they were transferred to boards where they were held in place by pieces of cork. The boards were placed upright in a large galvanized iron container, under a spray. They were kept in this position for at least 24 hours, and then exposed to gravity by rotating the board through 90°.

In the determination of moisture the corn nodes were dried to constant weight in vacuo at 80° C. The samples varied in weight from 2 to 5 gm., according to the number and size of the nodes used. Table I gives the results of the series in which the

nodes were exposed to gravity for varying lengths of time, from 3 to 27 hours. In the last column, + is in favor of the convex side and − in favor of the concave. This method of statement is used in all the tables. As already mentioned, corn nodes are not at all uniform in their response to gravity, and because of this fact a second set was run in which nodes that had bent approximately to the degree indicated were used. The results will be found in table II.

TABLE II

MOISTURE AND ACIDITY IN CORN NODES EXPOSED TO GRAVITY

DEGREE OF BENDING	MOISTURE			ACIDITY IN CC. 0.05 *N* NaOH PER GM. FRESH WEIGHT		
	Upper flank (percentage)	Lower flank (percentage)	Difference (percentage)	Upper flank	Lower flank	Difference
0.....	82.41	81.30	−1.11	0.62	0.65	+0.03
5.....	80.27	80.08	−0.19	0.72	0.75	+0.03
5.....	81.19	80.44	−0.75	0.60	0.73	+0.13
5.....	84.68	84.21	−0.47	0.60	0.63	+0.03
10.....	80.42	80.77	+0.35	0.75	0.83	+0.08
10.....	86.04	86.35	+0.31	0.56	0.60	+0.04
15.....	86.31	87.63	+1.32	0.65	0.72	+0.07
20.....	85.13	87.60	+2.47	0.66	0.80	+0.14
25.....	87.12	89.52	+2.40	0.80	0.76	−0.04
25.....	87.52	89.29	+1.77	0.66	0.71	+0.05

Individual differences in moisture content are so great that different samples cannot be compared. It is only possible to compare opposite flanks of the same sample. In general the differences are slight, and in view of the high percentage of moisture present they may not be significant. There are some features of the results which are of interest, however, especially when the two sets are compared. In the time of exposure set the differences are variable, but in general favor the convex side up to 9 hours of exposure. At 12 and 15 hours, when bending is well started, there is a decided difference in favor of the concave side. At 18, 21, and 27 hours the convex side contains much more moisture. The results at 24 hours appear to be anomalous, especially as no corresponding change is found in the other set. In the degree of bending set the differences are more regular and more marked. During the early stages of bending the concave flank contains the more moisture, but

as bending proceeds the convex flank contains more water. The same difference is indicated in the time of exposure set, but because of irregularities in the response of the nodes, it is not so obvious.

The results with *Vicia Faba* shoots are given in table III. The fresh samples weighed about 1 gm. They were dried to constant weight at 100–102° C. The differences are so small and so

TABLE III

MOISTURE AND ACIDITY IN *Vicia Faba* SHOOTS EXPOSED TO GRAVITY

TIME OF EXPOSURE	MOISTURE			ACIDITY IN CC. 0.05 *N* NaOH PER GM. FRESH WEIGHT		
	Upper flank (percentage)	Lower flank (percentage)	Difference (percentage)	Upper flank	Lower flank	Difference
15 minutes...	93.35	93.35	.....	1.40	1.15	−0.25
15 minutes...	93.33	93.33	.....	1.18	1.18	.....
30 minutes...	92.43	92.50	+0.07	1.10	1.15	+0.05
30 minutes...	93.25	93.13	−0.12	0.99	1.05	+0.06
45 minutes...	92.48	91.63	−0.85	1.05	1.12	+0.07
45 minutes...	92.67	92.73	+0.06	1.07	0.94	−0.13
1 hour.....	93.02	93.19	+0.17	1.16	1.19	+0.03
1 hour.....	92.40	92.50	+0.10	1.20	1.16	−0.04
2 hours.....	91.53	91.50	−0.03	1.54	1.58	+0.04
2 hours.....	93.00	93.03	+0.03	1.39	1.37	−0.02
3 hours.....	92.50	92.65	+0.15	1.18	1.20	+0.02
3 hours.....	92.13	92.45	+0.32	1.15	1.10	−0.05
5 hours.....	92.50	92.80	+0.30	1.23	1.17	−0.06
5 hours.....	92.95	93.15	+0.20	1.18	1.10	−0.08
7 hours.....	92.70	92.63	−0.07	1.02	0.91	−0.11
7 hours.....	92.60	92.60	.....	1.13	1.08	−0.05
9 hours.....	92.37	92.70	+0.33	1.13	1.14	+0.01
9 hours.....	92.87	92.93	+0.06	1.25	1.22	−0.03
11 hours.....	92.35	92.00	−0.35	1.13	1.11	−0.02
11 hours.....	92.65	92.65	.....	1.13	1.19	+0.06
13 hours.....	92.69	92.80	+0.11	1.15	1.12	−0.03
13 hours.....	92.87	92.73	−0.14	1.15	1.12	−0.03
17 hours.....	92.97	92.89	−0.08	1.13	1.26	+0.13
17 hours.....	92.25	92.27	+0.02	1.32	1.41	+0.09
21 hours.....	91.60	91.60	.....	1.15	1.11	−0.04
21 hours.....	93.00	93.07	+0.07	0.97	0.95	−0.02

irregular as to be insignificant. At the periods from 1 to 9 hours the convex side seems to contain, in general, a little more moisture, but the differences are too slight to serve as a basis for any conclusions.

For the determination of titration acidity the samples were ground in a mortar with sand which had been treated with HCl and washed free from acid. Fifty cc. of water was added and the mixture titrated to phenolphthalein with 0.05 *N* NaOH. Blanks

were run on the sand and water, and were used to correct the results. There was not enough color in the material to interfere seriously with the phenolphthalein endpoint, but the endpoint is somewhat slow, and, especially with material containing so little acid, the unavoidable errors are apt to cause differences which represent a large percentage of the total titration. The results for corn nodes, calculated as cubic centimeters 0.05 *N* NaOH per gram of fresh material, are given in tables I and II. The differences found between the two flanks are small. The convex side seems quite uniformly to be the more acid.

A few measurements of the hydrogen ion concentration of the press juice of corn nodes which had bent from 5° to 15° were obtained. The measurements were made electrometrically, using a modified form of the Barendrecht electrode. The following P_H values were obtained, that for the upper flank being given first in each case: 4.919, 5.012; 5.136, 5.246; 5.104, 5.198. In these three cases, therefore, the hydrogen ion concentration of the juice of the concave flank was the greater, although, as has been noted, the titration acidity varied quite uniformly in the other direction.

The titration results with *Vicia Faba* are given in table III. The differences are slight and irregular, and do not correspond at all closely with those reported by Miss SCHLEY.

Determinations of hydrogen ion concentration, and electrometric titrations, were made on the press juice of the upper and lower flanks of *Vicia Faba* seedlings that had been exposed to gravity. The material was frozen immediately after collection. A special hand press was used which would remove the juice very completely from samples containing not more than 10 gm. of the fresh material. Five cc. of the juice was taken for the determination. The hydrogen ion concentration was determined immediately, after adding 1 cc. of 0.10 *N* NaOH free from carbonates. This is practically the method used by EMSLANDER (4) in his work with beer. Preliminary experiments showed that the part of the titration curve including these two points is always, for this material, the straight line part of the curve which crosses the neutral line. Usually the two points obtained were on opposite sides of neutrality, so that the cubic centimeters of 0.10 *N* NaOH required to titrate to $P_H = 7.0$

could be calculated by interpolation. In only one case was it necessary to extrapolate.

In table IV are given the P_H values of the press juice, and the cubic centimeters of 0.10 *N* NaOH required to bring 5 cc. of the juice to the neutral point. The results obtained on right and left halves of seedlings not exposed to gravity are given in the last two lines of the table. These results show the magnitude of the differences that might arise from other causes than the action of gravity, such as actual differences between two sides of a plant, and errors in measurement. In a few cases the differences found

TABLE IV

ELECTROMETRIC DETERMINATIONS ON PRESS JUICE OF *Vicia Faba* SHOOTS EXPOSED TO GRAVITY

TIME OF EXPOSURE	HYDROGEN ION EXPONENT			ACIDITY IN CC. 0.10 *N* NaOH PER 5 CC. OF JUICE		
	Upper flank	Lower flank	Difference	Upper flank	Lower flank	Difference
30 minutes...	6.124	6.198	+0.074	0.81	0.77	−0.04
30 minutes...	6.122	6.060	−0.062	0.89	1.05	+0.16
1 hour.....	6.127	6.207	+0.080	0.81	0.71	−0.10
1 hour.....	6.137	6.092	−0.045	0.83	0.92	+0.09
2 hours.....	6.144	6.198	+0.054	0.75	0.77	+0.02
2 hours.....	6.132	6.160	+0.028	0.79	0.75	−0.04
4 hours.....	6.203	6.060	−0.143	0.74	0.81	+0.07
4 hours.....	6.170	6.193	+0.023	0.72	0.75	+0.03
Not exposed.	6.079	6.102	+0.023	0.88	0.82	−0.06
Not exposed.	6.048	6.103	+0.055	0.87	0.79	−0.08

between the flanks of plants acted on by gravity are greater than those in the blank determinations, but where this is the case the differences are not regular in direction.

The plan of the work included as complete a study as possible of the various oxidizing enzymes. Only the catalase had been studied when it became necessary to discontinue the work. Determinations of catalase activity were made by the method of APPLEMAN (1), as modified and used by CROCKER and HARRINGTON (2). Catalase activity decreases from the tip downward, and it is not exactly proportional to the weight of the sample. It was not possible entirely to avoid the errors from both of these sources. The following method was used. After exposure to gravity the

shoot was divided as accurately as possible into upper and lower flanks. A sample was cut from one of the flanks, starting at the tip and going as far as was necessary to obtain exactly 0.200 gm. The other flank was left attached to the plant, and kept in a moist dark place while catalase was determined in the first sample. The second flank was then sampled in the same way as the first, and its catalase content determined. Six plants were used for each period of exposure. The catalase content of the upper flank of three of these was determined first, that of the lower flank of the other three first. The 0.200 gm. sample was ground for 2 minutes in a mortar with sand and a little $CaCO_3$. It was then washed into the apparatus with 15 cc. of water. After the apparatus had reached the temperature of the bath, 5 cc. of H_2O_2 (dioxygen), neutralized with a little $CaCO_3$, was added. Shaking was begun at once, and readings of the volume of oxygen evolved were taken every minute for 10 minutes. The bath was kept at 25° C. and the air temperature did not change significantly during any single set of determinations.

The results given in table V are the cubic centimeters of oxygen evolved in 10 minutes. The average of the results for each of the periods of exposure is in favor of the upper flank, but only in the case of the 1 hour samples were all the results in this direction. In the other sets the individual results vary so widely that no conclusions can be drawn from the averages.

For chemical analysis samples of about 100 gm. fresh weight were used. These were collected in flasks containing 0.5 gm. $CaCO_3$ and sufficient alcohol so that the final concentration was approximately 80 per cent. It was during the collection of the last of these samples that it became necessary to drop the work. In order that the material might not be lost, H. A. JONES consented to complete the collection and carry out the analyses. The writer wishes to express his thanks to Dr. JONES for his kindness in making this addition to the data possible.

The soluble and insoluble portions were separated, and total solids determined in each. Sugars were determined as follows. Aliquots of the extract were evaporated to remove alcohol, taken up with water, and clarified with basic lead acetate. The excess lead

was removed by Na_2SO_4. In the filtrate reducing sugars were determined before and after subjecting it to the standard method for the hydrolysis of sucrose by HCl. The Bertrand titration method was used for determining the amounts of copper reduced. The results are expressed as glucose and sucrose respectively, although it is recognized that other sugars are undoubtedly included. Total nitrogen was determined in both the soluble and insoluble

TABLE V

CATALASE ACTIVITY IN SHOOTS OF *Vicia Faba* EXPOSED TO GRAVITY (EXPRESSED AS CUBIC CENTIMETERS OF OXYGEN LIBERATED BY 0.20 GM. OF MATERIAL)

Time of exposure	Upper flank	Lower flank	Difference
30 minutes	7.15	7.80	+0.65
30 minutes	8.20	8.00	−0.20
30 minutes	8.45	7.50	−0.95
30 minutes	8.40	6.30	−2.10
30 minutes	9.00	10.70	+1.70
30 minutes	7.10	6.70	−0.40
1 hour	9.85	8.65	−1.20
1 hour	9.40	8.20	−1.20
1 hour	12.20	11.40	−0.80
1 hour	10.20	9.00	−1.20
1 hour	8.00	7.50	−0.50
1 hour	8.80	8.70	−0.10
2 hours	9.85	10.05	+0.20
2 hours	8.80	9.00	+0.20
2 hours	10.10	9.95	−0.15
2 hours	11.30	11.00	−0.30
2 hours	7.10	7.40	+0.30
2 hours	8.60	7.60	−1.00
4 hours	9.25	9.30	+0.05
4 hours	7.40	7.10	−0.30
4 hours	5.15	6.60	+1.45
4 hours	8.80	8.50	−0.30
4 hours	7.10	6.60	−0.50
4 hours	8.65	7.60	−1.05

portions by the Kjeldahl method. The results are given in table VI. The differences in direct reducing sugars, "glucose," are comparatively slight. Those in reducing sugars formed on hydrolysis, "sucrose," are considerably greater, especially when figured as percentages of the total. It is to be remembered, however, that the total amount of sucrose is relatively small, and that the errors in both determinations may accumulate in that of sucrose. It seems

to be impossible to correlate the differences found with the process of bending. The same may be said of the distribution of nitrogen.

Summary

Definite moisture changes accompany geotropic bending in corn nodes. During the early stages of bending there is a greater percentage of moisture in the concave flank. When the process has developed the percentage of water is greater in the convex flank.

Although titration acidity is greater in the convex flank, the differences are very slight. The results on hydrogen ion concentration, although uniform in direction, are not numerous enough to serve as a basis for conclusions.

It is impossible, with the data obtained, to correlate the geotropic bending of etiolated *Vicia Faba* shoots with differences in

TABLE VI

ANALYSES OF *Vicia Faba* SHOOTS EXPOSED TO GRAVITY (IN PERCENTAGE OF FRESH WEIGHT)

Time of Exposure	Upper flank	Lower flank	Difference	Upper flank	Lower flank	Difference
	Glucose			Sucrose		
30 minutes...	2.16	2.15	−0.01	0.450	0.279	−0.171
1 hour.....	1.47	1.40	−0.07	0.187	0.287	+0.100
2 hours.....	1.56	1.67	+0.11	0.221	0.269	+0.048
4 hours.....	1.37	1.41	+0.04	0.449	0.289	−0.160
	Total sugars			Moisture		
30 minutes...	2.61	2.43	−0.18	91.57	92.39	+0.82
1 hour.....	1.65	1.69	+0.04	92.54	92.46	−0.08
2 hours.....	1.78	1.94	+0.16	91.62	91.82	+0.20
4 hours.....	1.82	1.70	−0.12	91.93	92.13	+0.20
	Soluble nitrogen			Insoluble nitrogen		
30 minutes...	0.294	0.301	+0.007	0.302	0.284	−0.018
1 hour.....	0.261	0.259	−0.002	0.303	0.306	+0.003
2 hours.....	0.279	0.305	+0.026	0.314	0.296	−0.018
4 hours.....	0.264	0.258	−0.006	0.349	0.324	−0.025

moisture, titration acidity, hydrogen ion concentration, catalase activity, or the distribution of sugars and nitrogen containing substances.

The writer wishes to express his thanks to Dr. WM. CROCKER for his continued interest in the work, and for his many helpful suggestions.

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POSITION OF SCUTELLUM AND HOMOLOGY OF COLEOPTILE IN MAIZE

(WITH ELEVEN FIGURES)

PAUL WEATHERWAX

The homologies of the grass embryo and their bearing upon ideas of the phylogenetic relationship of monocotyledonous and dicotyledonous plants have been subjects of study and discussion for a long time, and although most botanists are fairly well agreed upon most phases of the question, some points are still subject to controversy. It is realized that evidences drawn from a single species as highly specialized as maize will not go far toward the making or the breaking of a theory, but two things have been observed in the structure and development of the embryo of *Zea Mays* that seem to have a definite bearing upon the subject, and these are offered for what they may be worth.

The history of the subject has been fully reviewed, and certain sharply contrasted opinions have been presented recently by WORSDELL and by COULTER and LAND. Further reference to the voluminous literature seems unnecessary here, and only those points to which the information at hand is related will be considered.

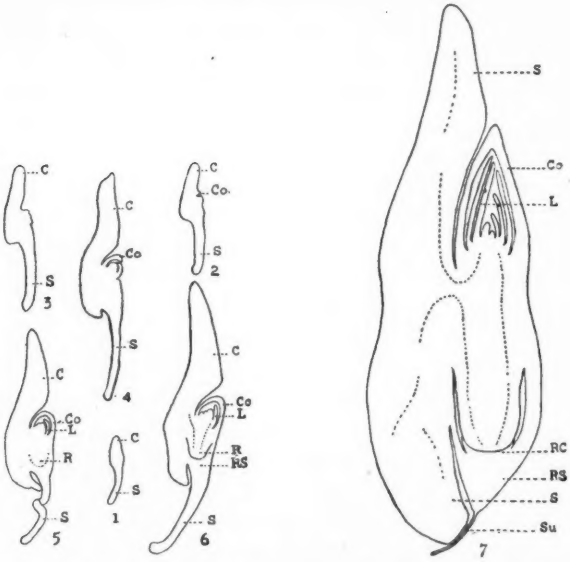
COULTER and LAND[1] maintain that the scutellum of the grass embryo is a lateral organ, the equivalent of the foliage leaf. The epiblast represents the cotyledon that was lost in the evolution from the monocotyledonous condition, and the coleoptile is the third leaf. Opposed to this is WORSDELL'S[2] contention that the cotyledon, which he considers terminal in origin, is the lamina, and the coleoptile is the ligule, of a single foliage leaf, whose sheath was present only in early stages of development. The epiblast is said to be the equivalent of the auricles of the foliage leaf. The principal evidences brought to the support of this view are the double

[1] COULTER, J. M., and LAND, W. J. G., The origin of monocotyledony. II. Monocotyledony in grasses. Ann. Mo. Bot. Gard. 2:175–183. 1915.

[2] WORSDELL, W. C., The morphology of the monocotyledonous embryo and that of the grass in particular. Ann. Botany 30:509–524. 1916.

nature of the vascular system of the coleoptile, the bifid character of the epiblast in some grasses, and the forked coleoptile found in a few seedlings of maize. As a background for these details is the idea that the monocotyledonous condition is the primitive one.

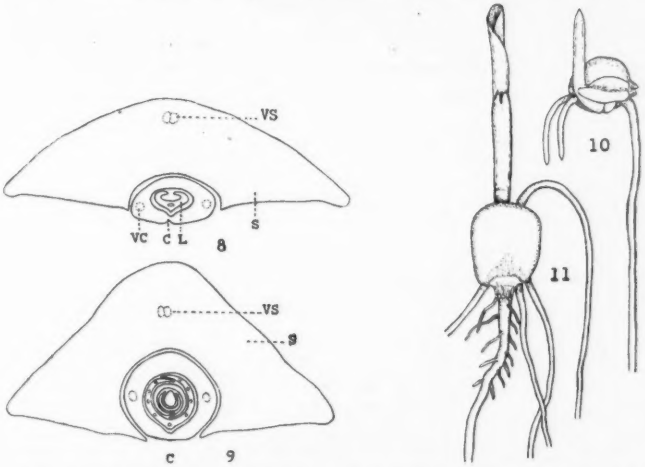
The first piece of evidence that I have to offer on these questions is in the form of a series of steps in the development of the embryo of maize (figs. 1–7). These stages have often been observed and



FIGS. 1–7.—Figs. 1–6, steps in development of embryo: *C*, cotyledon; *S*, suspensor; *Co*, coleoptile; *L*, foliage leaf; *R*, root; *RS*, root sheath; ×15; fig. 7, longitudinal section of nearly mature embryo: *S*, scutellum; *Co*, coleoptile; *L*, foliage leaf; *RC*, root cap; *RS*, root sheath; *Su*, suspensor; ×15.

discussed more or less abstractly, but I have failed to find a complete series figured. In so far as appearances may be trusted, no evidence is clearer than this series. The appearance of the mature embryo (fig. 7) leaves little doubt of the terminal position of the plumule, and preceding stages of development bear this out fully; the cotyledon is never terminal, even in the earliest stages. As soon as the young embryo begins to differentiate, so that anything

that may be called a cotyledon is visible (fig. 1), the whole structure has an asymmetrical form due to the more rapid development laterally of the cotyledon, and subsequent steps emphasize this (figs. 2–7). That the coleoptile is at first directed horizontally or downward, as is emphasized by WORSDELL, is of little significance; morphological position cannot always be determined geometrically. Moreover, WORSDELL'S[3] figures, taken from another authority in



FIGS. 8–11.—Figs. 8, 9, transverse sections of embryo through plumule: *VS*, vascular strands of scutellum; *VC*, vascular strands of coleoptile; *L*, foliage leaf; *C*, point of union between two sides of coleoptile, forming closed sheath; sections of embryo of liguleless variety in no essential way different from these; ×15; fig. 10, germinating seed of liguleless maize: coleoptile present and normal; fig. 11, seedling of maize, showing forked coleoptile.

substantiation of his position, are only the upper parts of embryos; if we attach to the figures the lower parts of the corresponding stages of development of the embryo of any typical grass, the continuity of cotyledon, hypocotyl, and suspensor as the axis of the embryo is evident.

The second point in support of the view taken by COULTER and LAND is afforded by the embryo of a liguleless variety of maize isolated by EMERSON a few years ago. These plants are like those of ordinary maize, except that they breed true for the absence of

[3] *Ibid.*, fig. 3, A-E, p. 511.

ligules and auricles. A few of the plants tend to produce at least rudimentary ligules on the uppermost leaves, but they are regularly absent from the lower leaves, and the condition might reasonably be expected to extend to the cotyledon also. An examination of the embryo and of the seedling, however, shows the coleoptile to be present and normally developed (figs. 8–10). While this fact cannot be accepted as a proof of anything, it should at least not be overlooked in considering the question.

WORSDELL has probably given undue emphasis to the arrangement of the vascular strands of the coleoptile and to the forked tip of this organ in some seedlings of maize (fig. 11). It is true that the coleoptile has two vascular strands bilaterally placed (figs. 8, 9), while the foliage leaf has several strands equally distributed; but this modification in vascular anatomy is no more significant than that shown in the scutellum (figs. 8, 9), in a prophyllum, or in the palea of many grasses, all of which tend to have their vascular elements present in two groups, and yet all of which are considered modified foliage leaves. The forked coleoptile is a common occurrence often noted by anyone having occasion to examine a large number of seedlings of maize, and it is due to a superficial set of conditions. The coleoptile begins to develop as an open sheath (figs. 2–4), the edges of which soon unite to form a closed structure; but the line of this union is always visible (figs. 8, 9), especially near the top of the sheath. Too much significance must not be attached to the nature of the mechanical rupture of this structure by the elongating plumule. If the union of the two sides has not been very firm, and it usually is not, the structure will split on one side only; but if the two sides are firmly grown together, the coleoptile may split for a short distance down two sides, producing the forked coleoptile (fig. 11). The relation between this occurrence and the duplex ligules of some grasses, or the two stipules of some other plants, is too remote to merit consideration.

It may be said, therefore, that the evidences derived from the structure and development of the maize embryo, including that of the liguleless mutant, favor the idea that the coleoptile is the homologue of a foliage leaf, and that the cotyledon is a lateral organ.

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CURRENT LITERATURE

NOTES FOR STUDENTS

Ecological terms and concepts.—Ecology, one of the latest branches of botanical science, has naturally an immature and incomplete terminology, although several of its followers have made attempts to remedy the latter defect. Some of the recent discussions of problems of terminology are worthy of note, not so much for their contributions to nomenclature, as to their logical division of the subject and their criticism of the mistakes of the past. This seems particularly true of an article by PAVILLARD,[1] in which he begins with a historical and critical sketch of conditions in the past, and proceeds with an analysis of the scope of plant geography. Here it is suggested that it would be desirable for all to follow the practice of the Swiss school and employ the designation "geobotany" suggested by GRISEBACH in 1866. Two main divisions of the science are then made, resting upon the two fundamental units of the species and the association. It is further suggested that the latter be appropriately termed plant sociology or phytosociology, and that the problems of each division be segregated into three categories, giving as the subdivisions of the subject: (1) Floristic geobotany, (2) Genetic geobotany, (3) Ecologic geobotany, (4) Floristic phytosociology, (5) Genetic phytosociology, and (6) Ecologic phytosociology. Whether this classification be universally adopted or not, it has much to recommend it in logical clearness, and, further, it shows considerable agreement with the best usage of the past.

The content of floristic geobotany would remain the same as for floristic plant geography as delimited by WARMING in 1895, while ecologic geobotany would not differ materially from the autecology of SCHRÖTER in giving emphasis to the relationship of the individual species to its habitat and the growth form by which it responds to its environment. To genetic geobotany would be referred such questions as the geographical aspect of the origin of species, heterogenesis, and endemism.

In the division devoted to problems connected with the plant association, the use of the term phytosociology or plant sociology was proposed by JACCARD in 1910, and employed in a more limited sense by HARPER.[2] The second and third subdivisions seem about equivalent to SCHRÖTER's synecology, genetic

[1] PAVILLARD, J., Les progrès de la nomenclature dans la géographie botanique. Ann. Geogr. **27**:401–415. 1918.

[2] HARPER, R. M., The new science of plant sociology. Sci. Monthly **4**:456–460. 1917.

phytosociology being almost exactly the same as COWLES's[3] physiographic ecology, while ecologic phytosociology corresponds very closely to WARMING'S ecological plant geography. To floristic phytosociology would be referred not only the enumeration of the flora of the associations, but also more exact studies as to the importance of the species to the community, and the constancy of the relationship. These relationships are discussed in detail by PAVILLARD[4] in a more recent paper. One phase of this relationship has been estimated in a quantitative manner by BRAUN-BLANQUET,[5] by giving to each species in an association a coefficient of affiliation (Gesellschaftstreue), the first rank (5) being conferred upon species confined exclusively to the particular association, and the lowest (0) belonging to ubiquists. To this PAVILLARD adds another evaluation of the species, based upon its importance in development and maintenance of the association, and expressed as its genetic coefficient, and here also the numerical value is also from 5 to 0. Analyzed in such a manner, the floristic composition seems to PAVILLARD decidedly the best manner of characterizing an association. The characterization of the plant association by floristic composition only is also insisted upon by DU RIETZ and his associates.[6] They also favor attention to priority in the use of ecological terminology, a concession that ecological writers are not likely to grant. DU RIETZ contends that the Swedish school of ecologists is distinguished by the use of true inductive methods as contrasted with the less desirable procedure of other workers. He also proposes certain new terms of minor importance.

GAMS[7] is less modest in his demands, for he wishes to abolish the use of formation, association, and most other synecological (or biocoenological) terms now current, because they have been and still are being employed in different senses by different writers. Instead of such fairly familiar terms, he would substitute a new set founded to some extent on new concepts. He contends that two types of units, the ecological and topographical, have been confused and should be distinguished with care. The former he calls "synusia" (associations), and distinguishes three grades where the component elements

[3] COWLES, H. C., The physiographic ecology of Chicago and vicinity. BOT. GAZ. 31:73–86. 1901.

[4] PAVILLARD, J., Remarques sur la nomenclature phytogéographique. Montpellier. pp. 27. 1919.

[5] BRAUN-BLANQUET, J., Eine pflanzengeographische Excursion durch Unterengadin und in dem schweizerischen National Park. Bericht. Schw. Bot. Gesells. 26:1–79. 1918.

[6] DU RIETZ, C. E., FRIES, T. C. E., and TENGWALL, T. A., Vorschlag zur Nomenklatur der soziologischen Pflanzengeographie. Svensk. Bot. Tidskrift 12:145–170. 1918.

[7] GAMS, H., Prinziprenfragen des Vegetationsforschung. Ein Betrag zur Begriffsklarung und Methodik der Biocoenologie. Vierteljahrschr. Naturf. Gesells. in Zurich 63:293–493. 1918.

of the unit are respectively (1) of the same species, (2) of different species but of the same growth forms and of similar aspect, and (3) of different species and various growth forms presenting different series of aspects but united into an ecological unit in a single habitat by fixed correlation. This last grade of synusium corresponds very nearly with the "association" of most authors. Similar synusia are grouped as "isocies." For the topographical unit he adopts the word "biocoenose" (or biocoenosium), and uses it for the vegetation of a unit habitat. Biocoenosia of different regions which are compounded of isocies are called "isocoenosia."

The author rejects all attempts to classify vegetation units upon dynamic lines. He also gives a new classification of life forms, based largely upon the RAUNKIAER system, but more extended and including animals. It is safe to predict that such revolutionary changes as those urged by GAMS, even if they are logically conceived, will not be acceptable to the ecologists of America, and, judging from the criticism of the scheme by PAVILLARD (1919), they will meet with no greater favor in France.—GEO. D. FULLER.

Statistical methods in ecology.—It seems appropriate that from among the students of that father of modern ecology, EUGENE WARMING, should come a leader of perhaps the most promising line of advance in the ecology of today. RAUNKIAER more than any other has opened the way for the introduction of quantitative methods in the study of vegetation. His method of comparing the floras of different regions by means of a numerically expressed biological spectrum,[8] and of evaluating the mesophytism of a habitat by leaf classes,[9] have been noted in this journal. The latter method of estimating vegetation was made more familiar to American ecologists by the translation of FULLER and BAKKE,[10] who also included in their article a summary of a statistical method that had been familiar to Danish readers for some years.[11]

In a more recent article RAUNKIAER[12] has summarized the material of his former contributions, and has been able to show something of their applications to the solution of ecological problems. His statistical or valence method consists in determining the relative abundance of the different species composing a plant community of definitely limited extent, called by him a "formation," although more nearly equivalent to an association as understood by American ecologists. This determination is made by taking a census of a

[8] BOT. GAZ. **51**:309–310. 1911.

[9] BOT. GAZ. **63**:242. 1917.

[10] FULLER, GEO. D., and BAKKE, A. L., Raunkiaer's life-forms, leaf-size classes, and statistical methods. Plant World **21**:25–37, 57–63. *fig. 1*. 1918.

[11] RAUNKIAER, C., Formations undersgelse og Formationsstatistik. Bot. Tidskr. **30**:20–132. 1909.

———, Om Valensmetoden. Bot. Tidskr. **34**:304–311. 1917.

[12] ———, Recherches statistiques sur les formations végétales. Det. Kgl. Danske Videnskabernes Selskab. Biol. Meddeleser. I **3**: pp. 80. *figs. 3*. 1918.

number (25–50) of small unit areas of the vegetation, selected at random or according to fixed plans, and outlined by the revolution of a metal radius of determined length attached to a walking stick.[13] The convenient size of these unit areas appears to be 0.1 sq. m., and the frequency with which a given species appears in such areas determines its valence, frequency percentage, or frequency coefficient. Emphasis is placed upon the fact that in an undisturbed area the vegetation will eventually come to a practically complete equilibrium with the factors of the habitat, and will be composed of the species of the region best fitted to exist under such conditions. RAUNKIAER therefore defines his "formation" as "essentially homogeneous from a floristic point of view," that is, homogeneous as to the dominant species or the species showing the highest frequency coefficients. Such a statistical method permits the quantitative comparison of similar plant communities and their more exact delimination.

It is interesting to note as the results of the use of such statistical methods, principally the examination of many plant communities involving the determination of over 8000 coefficients, that 55 per cent of the species have coefficients ranging from 1 to 20, 15 per cent from 81 to 100, 14 per cent from 21 to 40, 9 per cent from 61 to 80, and 3 per cent from 41 to 60. In other words, the least frequent species in the communities studied were most numerous, while the most frequent came second in order of number of species, with a much smaller number showing moderate abundance. These phenomena the author expresses in the form of a law. "In a formation in a relative state of equilibrium what allows one or more species to prosper at the expense of their neighbors is the fact that the dominant species are better adapted to live under the conditions existing within the formation of which they are a part and by their community life ('concurrence vitale') they prevent the other species from equaling them in frequency. But however well equipped they may be for such community life, they are not able to prevent other species, widely disseminated but fewer in individuals, from entering the formation and occupying portions that for any reason whatever may have been left unoccupied by the dominant species. Thus we see that there is a much larger number of the least frequent species."

For the further analysis of vegetation RAUNKIAER describes a method of arriving at the area occupied by each species in the community. This is accomplished by the study of unit areas similar to those employed in the determination of frequency; indeed the two could be done simultaneously. To assist in readily determining the portion of the area occupied by the areal parts of a species he adds a series of radii of determined length to the one already affixed at right angles to the walking stick. These are so spaced that they divide the circular unit area into fifths and tenths, so that by their aid

[13] RAUNKIAER, C., Measuring apparatus for investigations of plant formations. Bot. Tidskr. 33:45–48. 1912.

the observer is easily able to estimate 10 different degrees of covering. From a record of the numbers representing these degrees of covering the areal percentages of the different species are readily established.

A summary of the methods employed, and a classification of vegetation upon the basis of life-forms and leaf-sizes, completes an article rich in suggestions to the ecologist seeking more accurate methods.—GEO. D. FULLER.

Susceptibility gradients.—Following his demonstration of axial metabolic gradients in animals and their relation to the course of development and individuation, CHILD entered upon a study of axiate plants, particularly the algae. His first paper[14] on axial gradients in algae appeared several years ago. His interesting and valuable observations[15] have been extended to include a considerable number of new forms, and the results are sufficiently uniform to warrant the general conclusion that plants and animals are essentially similar in respect to these axial susceptibility gradients.

Twenty-five species have been studied, 14 of which were considered in the earlier paper, and all of them show an axial gradient in susceptibility to injury and death from such agents as KCN, alcohol, ether, HCl, $HgCl_2$, $CuSO_4$, neutral red, temperature, etc. When killing concentrations are used, death occurs first in the apical region and proceeds basipetally in each axis. The susceptibility gradient is a general indicator of metabolic rates, death occurring soonest in the most active protoplasm. The susceptibility gradient is rather easily altered or reversed by external conditions, by advancing age, physiological isolation of cells and branches, and other factors. The ease with which such reversals occur indicates in some degree the sensitiveness of species.

He finds[16] that the unicellular and multicellular hairs, either branched or unbranched, which occur on some algae, possess the same kind of axial gradients as the main axis. In such forms as *Fucus* and *Castagnea*, in which the hairs have basal growth, the gradient is acropetal; but whenever the hairs grow apically the normal gradient is basipetal. Reversals may be induced in these hairs, also, especially with low concentrations of the susceptibility reagents. In some cases the agent may reverse the susceptibility to itself, or one agent may reverse the susceptibility to another agent. These results indicate clearly that hairs represent physiological axes, and the gradient of susceptibility appears to be one of the aspects of physiological polarity in all axes. When the axial gradients are reversed, these hairs often separate into their component cells, or the hairs drop from the main axes. Loss of hairs in laboratory material

[14] CHILD, C. M., Axial susceptibility gradients in algae. BOT. GAZ. **62**:89–114. 1916.

[15] ———, Further observations on axial susceptibility gradients in algae. Biol. Bull. **31**:419–440. 1916.

[16] ———, Susceptibility gradients in the hairs of certain marine algae. Biol. Bull. **32**:75–92. 1917.

is undoubtedly associated with reversed gradients brought about by unfavorable conditions of confinement.

These changes in gradients of hairs were studied particularly in *Griffithsia*.[17] If conditions are not extreme, obliteration or reversal of the axial gradient is followed by cell separation, and the death of some of the cells, the death-rate being higher among isolated apical cells than among those more basally situated. The cells which do not die usually proceed to grow new apical cells, which are found to arise at the most susceptible end of the old cells. This is usually the *basal* end, because the normal gradient had been reversed before the cells were disconnected. Rhizoids, however, arise only on those parts of the cell which have the lowest metabolic rates or lowest susceptibility.

The general conclusion of all this work is summarized admirably in the words of the author: "The facts support the conclusion that a gradient in metabolic rate, protoplasmic condition, or whatever we prefer to call it, of which the susceptibility gradient is within certain limits an indicator, constitutes physiological polarity in protoplasm, and that such a gradient is not an inherent property of protoplasm, but may be determined and altered by external factors."

Students who desire to repeat some of these experiments for themselves will find a recent paper of interest.[18] The axial gradient may be very beautifully demonstrated colorimetrically by the use of dilute solutions of potassium permanganate. The protoplasm reduces the permanganate and takes on a brown color, which appears first and deepest in the most active regions. Concentrations of M/1000 to M/100,000 should be used for such experiments.—C. A. SHULL.

Biology and culture of the higher fungi.—Among recent contributions to our knowledge of this difficult subject is a paper by BOYER[19]. The first part deals with attempts at spore germination and culture of over 60 species, and the second gives in more detail the results of his work with *Morchella* and *Psaliota*.

He recognizes three types of higher fungi: (1) pure saprophytes, (2) facultative parasites, and (3) mycorhizal forms which are constantly associated with certain trees. Saprophytes, he finds, grow readily on culture media, and many give rise to carpophores; while many of the mycorhizal group cannot be grown in pure cultures on any of the many types of media tried. Between pure saprophytes and forms which will not grow on culture media he finds

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[17] CHILD, C. M., Experimental alteration of the axial gradient in the alga *Griffithsia Bornetiana*. Biol. Bull. **32**:213–233. 1917.

[18] ———, Demonstration of the axial gradients by means of potassium permanganate. Biol. Bull. **36**:133–147. 1919.

[19] BOYER, G., Études sur la biologie et la culture des champignons superieurs. pp. 116. *pls.* 4. *figs.* 20. Bordeaux. 1918.

gradations in dependence upon the mycorhizal habit. Some will make only a very slight mycelial growth in cultures, while others will form abundant mycelia, but never develop carpophores. Field experiments also confirm this mycorhizal dependence, but attempts to trace mycelium from carpophore to tree were seldom successful. He considers the mycorhizal relationship to be symbiotic, the green plant furnishing carbohydrates and in return receiving water and salts, especially nitrogenous substances which the fungi probably obtain by the fixation of free nitrogen.

As a source of cultures he first tried the germination of spores. Various media and methods of treating spores were tried, but no germinations from mycorhizal forms such as tubers or amanitas were obtained, and from other forms the mycelium obtained was seldom vigorous. Because of this he resorted to the use of portions of the carpophore, flamed over a Bunsen burner, as a source of cultures, and found this (which he erroneously considers a new process) much more satisfactory. In this manner he obtained cultures of 24 species which he describes, giving figures for 17 of them. While many media were used, he found a decoction from carrots, solidified with gelose (a gum derived from agar-agar), the most satisfactory. Cultural variations bring into question the validity of some specific characters, such as size, color, and characters due to substratum.

In his studies of *Morchella* cultures were obtained from single spores. The mycelium was very vigorous, growing well at 10–12° C. Sclerotia 0.5–4 mm. in diameter appear in 10–15 days. No conidia or ascocarps were formed. He attributes the absence of ascocarps either to the limited mycelial growth in cultures, or, as he considers more probable, to the necessity of a mycorhizal host prior to ascocarp formation.

Cultures obtained from the spores of *Psaliota* were always weak, while those from portions of the carpophore were very vigorous. From his pure cultures he easily developed successful commercial spawn. Cultures from one carpophore always developed carpophores with the same varietal characters as the original, which is a great practical advantage.—LEVA B. WALKER.

Identification of mahoganies.—To meet the need of some adequate method for distinguishing the different commercial timbers now classed as mahoganies, DIXON[20] has prepared (1) a concise working definition of the term mahogany, and (2) an anatomical key accompanied by detailed descriptions for the identification of some of the more common kinds by means of their microscopic characters. The constant increase in the number of species of mahogany-yielding trees in economic use, and the doubtful authenticity of many of the specimens derived from commercial sources, have made the construction of such a scheme of classification most difficult.

[20] DIXON, H. H., Mahogany, the recognition of some of the different kinds by their microscopic characteristics. Notes from the Bot. School, Trinity College, Dublin 3:58. *pls. 22–54.* 1919.

The first part of this preliminary paper discusses the many varied properties of these different woods, with regard to color, density, hardness, presence or absence of year-rings, pore-rings, size and contents of vessels, distribution of parenchyma, etc., and also the numerous contradictory definitions of mahogany to which these structural differences have given rise. To the general public and to the majority of woodworkers, mahogany is a reddish wood, generally with some distinct figure and texture, and valued in proportion to the beauty of its figure and the resistance of the wood to splitting and warping. Obviously such a definition is not sufficient. Reddish color and figure, both emphasized as distinct diagnostics of the original mahogany, *Swietenia mahogoni*, of course are essential, as also is the character described as "roeyness." According to DIXON, we may recognize as mahogany "all red or red-brown timbers in which the fibers of the adjacent layers cross each other obliquely, and so give rise to a play of light and shade on longitudinal surfaces ('roe'), greatly emphasizing the figure and conferring on the wood a freedom from splitting and warping." In addition, a mahogany should have scattered vessels, isolated or in small radial groups; the circumvasal parenchyma should be thin, and the medullary rays not more than 9 cells in width and under 2 mm. in height. In other respects the different woods designated by this name exhibit great structural variability.

The second part of the article presents the key and well written anatomical diagnoses of Western, African, Asiatic, and Australasian mahoganies. The 23 plates are from photomicrographs of transverse, radial, and tangential sections of the various woods, and are intended to show their distinct microscopic features.—LADEMA M. LANGDON.

Comparative salt absorption.—STILES and KIDD[21] have published two papers on the mechanism of salt absorption by disks of carrots and of potato tubers. Their method of study was to immerse a quantity of uniform disks of the material in salt solutions, and follow the course of absorption by the changes in the electrical conductivity. Although the conductivity is affected, not only by absorption of salt, but also by exosmosis, the writers believe that the latter is small, especially in the case of carrot. Potassium, sodium, and calcium chlorides are readily absorbed in all concentrations from N/10 to N/5000. The initial rate of absorption is roughly proportional to the concentration, but after a time this does not hold. The ratio of final internal concentration (arrived at by calculation) to final external concentration they call the absorption ratio. With low external concentrations this ratio is many

[21] STILES, W., and KIDD, F., The influence of external concentration on the position of the equilibrium attained in the intake of salts by plant cells. Proc. Roy. Soc. B **90**:448–470. 1919.

———, The comparative rate of absorption of various salts by plant tissue. Proc. Roy. Soc. B **90**:487–504. 1919.

times unity, but with higher concentrations it becomes considerably less than unity. Although this relation can be expressed by the adsorption formula $y=kc^m$ (y is the final internal, c the final external concentration, and k and m are constants), the writers do not feel the data justify the conclusion that absorption of these salts is an adsorption phenomenon.

Kations are absorbed initially in the order K, [Ca, Na], Li, [Mg, Zn], Al; as equilibrium is approached the order is K, Na, Li, [Ca, Mg]. The initial order for the anions is SO_4, NO_3, Cl; the final order, NO_3, Cl, SO_4. "Although TROENDLE's view, that in any group of the periodic classification the metallic ions are absorbed more rapidly the higher the atomic weight, is not contradicted, yet the view that the initial rate of absorption is largely dependent upon the mobility of the ions or diffusibility of the salt is equally well supported, and can be put forward provisionally as a more reasonable hypothesis."

Another paper, by STILES and JÖRGENSEN,[22] is polemical with THODAY, concerning the method of estimating the osmotic pressure of sap by the swelling or shrinkage of the tissue when immersed in salt solutions. Using sections of the root of the red beet, they found that they neither gained nor lost in weight in 0.40 N NaCl, and that this concentration was also just insufficient to cause plasmolysis. The writers therefore maintain that this concentration is *approximately* isotonic with the beet root sap.—J. J. WILLAMAN.

Tyrosin in fungi.—DODGE[23] reports some investigations on the chemistry of the tyrosinase reaction in the fungi which turn blue or black on exposure to air. The fungi were sliced, dried, and then ground into a flour, and this fungus flour used in the investigation. "In the work with tyrosin, the dried fungus flour was added directly to the substrate, toluol added, and the mixture left to extract the enzym and the enzym to react with the tyrosin." The author studied the reactions with the amino, carboxyl, and phenol groups. A modified form of the "micro" VAN SLYKE apparatus was used for the determination of the amino nitrogen, the permutite method of FOLIN and BELL for the determination of ammonia, and the colorimeter method of DUGGAR and DODGE for the determination of the carboxyl and phenol groups.

The following conclusions are drawn from these investigations: "(1) that the tyrosinase reaction is not a deamination, although it is possible that deaminases may exist in the same organism with tyrosinase; (2) that the tyrosin molecule is synthesized into a larger, more complex molecule, in which part of the carboxyl groups is either split off as carbon dioxide, or more probably bound in the molecule so that it will not react with alkali."—J. WOODARD.

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[22] STILES, W., and JÖRGENSEN, W., On the relation of plasmolysis to the shrinkage of plant tissue in salt solutions. New Phytol. **18**:40–50. 1919.

[23] DODGE, C. W., Tyrosin in the fungi: chemistry and methods of studying the tyrosinase reaction. Ann. Mo. Bot. Gard. **6**:71–92. 1919.

Cytology of gigantism.—The relation between the nuclei, and particularly the chromosomes, of exceptionally large individuals or varieties of a species has been described in several cases. TISCHLER[24] secured a giant form of *Phragmites communis* var. *Pseudodonax* which reached a height more than double that of the usual form. A comparison of the reduction divisions in the pollen mother cells of *P. communis* and the var. *Pseudodonax* brought him to the conclusion that, in this case, the gigantism arises through an increase in the size of the chromosomes, without any increase in their number. Other cases have been described in which the gigantism is due to an increase in the number of chromosomes, as in some forms of *Oenothera*, *Primula*, and *Solanum*.

The relation between chromosomes and dwarfing has received little attention from botanists, but the cytology of *Oenothera Lamarckiana* var. *nanella*, as described by GÁTES, and some observations by zoologists, indicate that the dwarfing is correlated, sometimes with a decrease in the number of chromosomes, and sometimes with a diminution in their size, without any change in their number.—C. J. CHAMBERLAIN.

Ecology of fossil plants.—In a report upon some fossil plant material found in the gorge of the Columbia River, in Oregon and Washington, CHANEY[25] notes that some 80 species are represented, 75 of which are angiosperms, of which 2 only are monocotyledons. A list of the genera with the number of species in each includes: *Ginkgo* 1, *Pinus* 1, *Smilax* 1, *Cyperacites* 2, *Populus* 3, *Salix* 3, *Hicoria* 2, *Juglans* 1, *Alnus* 1, *Carpinus* 1, *Corylus* 1, *Castanea* 1, *Quercus* 12, *Ulmus* 2, *Planera* 2, *Magnolia* 1, *Laurus* 2, *Platanus* 2, *Liquidambar* 3, *Crataegus* 1, *Sterculia* 1, *Rhus* 1, *Ilex* 1, *Acer* 3, and *Fraxinus* 1. From a study of this material the author concludes that the climate indicated by this Eagle Creek flora appears to have been somewhat warmer and drier than at present. The length of the epoch is to be placed at thousands rather than at scores of years. The dominant plants point to the existence of two habitats, one xerophytic and the other mesophytic. An area of upland dissected by a valley furnishes such habitats, and at the same time meets the geological requirements of the formation.—GEO. D. FULLER.

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[24] TISCHLER, G., Untersuchungen über den Riesenwuchs von *Phragmites communis* var. *Pseudodonax*. Ber. Deutsch. Bot. Gesells. **36**:549–558. *pl. 17*. 1918.

[25] CHANEY, R. W., The ecological significance of the Eagle Creek flora of the Columbia River gorge. Jour. Geol. **26**:577–592. *figs. 3*. 1918.